



PCT/AU2004/001051

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WITNESS my hand this  
Seventeenth day of August 2004

A handwritten signature in cursive script, reading "J. Billingsley".

JULIE BILLINGSLEY  
TEAM LEADER EXAMINATION  
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PROVISIONAL SPECIFICATION

**Applicant:**

BIONOMICS LIMITED  
A.C.N. 075 582 740

**Invention Title:**

MUTATIONS IN ION CHANNELS

The invention is described in the following statement:

MUTATIONS IN ION CHANNELS

Technical Field

5 The present invention is concerned with mutations in  
proteins having biological functions as ion channels and,  
more particularly, with such mutations where they are  
associated with diseases such as epilepsy and disorders  
associated with ion channel dysfunction including, but not  
restricted to, hyper- or hypo-kalemic periodic paralysis,  
10 myotonias, malignant hyperthermia, myasthenia, cardiac  
arrhythmias, episodic ataxia, migraine, Alzheimer's  
disease, Parkinson's disease, schizophrenia,  
hyperekplexia, anxiety, depression, phobic obsessive  
symptoms, neuropathic pain, inflammatory pain,  
15 chronic/acute pain, Bartter's syndrome, polycystic kidney  
disease, Dent's disease, hyperinsulinemic hypoglycemia of  
infancy, cystic fibrosis, congenital stationary night  
blindness and total colour-blindness.

20 Background Art

Epilepsies constitute a diverse collection of brain  
disorders that affect about 3% of the population at some  
time in their lives (Annegers, 1996). An epileptic seizure  
can be defined as an episodic change in behaviour caused  
25 by the disordered firing of populations of neurons in the  
central nervous system. This results in varying degrees of  
involuntary muscle contraction and often a loss of  
consciousness. Epilepsy syndromes have been classified  
into more than 40 distinct types based upon characteristic  
30 symptoms, types of seizure, cause, age of onset and EEG  
patterns (Commission on Classification and Terminology of  
the International League Against Epilepsy, 1989). However  
the single feature that is common to all syndromes is the  
persistent increase in neuronal excitability that is both  
35 occasionally and unpredictably expressed as a seizure.

A genetic contribution to the aetiology of epilepsy  
has been estimated to be present in approximately 40% of

affected individuals (Gardiner, 2000). As epileptic seizures may be the end-point of a number of molecular aberrations that ultimately disturb neuronal synchrony, the genetic basis for epilepsy is likely to be heterogeneous. There are over 200 Mendelian diseases which include epilepsy as part of the phenotype. In these diseases, seizures are symptomatic of underlying neurological involvement such as disturbances in brain structure or function. In contrast, there are also a number of "pure" epilepsy syndromes in which epilepsy is the sole manifestation in the affected individuals. These are termed idiopathic and account for over 60% of all epilepsy cases.

Idiopathic epilepsies have been further divided into partial and generalized sub-types. Partial (focal or local) epileptic fits arise from localized cortical discharges, so that only certain groups of muscles are involved and consciousness may be retained. However, in generalized epilepsy, EEG discharge shows no focus such that all subcortical regions of the brain are involved. Although the observation that generalized epilepsies are frequently inherited is understandable, the mechanism by which genetic defects, presumably expressed constitutively in the brain, give rise to partial seizures is less clear.

The molecular genetic era has resulted in spectacular advances in classification, diagnosis and biological understanding of numerous inherited neurological disorders including muscular dystrophies, familial neuropathies and spinocerebellar degenerations. These disorders are all uncommon or rare and have simple Mendelian inheritance. In contrast, common neurological diseases like epilepsy, have complex inheritance where they are determined by multiple genes sometimes interacting with environmental influences. Molecular genetic advances in disorders with complex inheritance have been far more modest to date (Todd, 1999).



Most of the molecular genetic advances have occurred by a sequential three stage process. First a clinically homogeneous disorder is identified and its mode of inheritance determined. Second, linkage analysis is performed on carefully characterized clinical populations with the disorder. Linkage analysis is a process where the chromosomal localization of a particular disorder is narrowed down to approximately 0.5% of the total genome. Knowledge of linkage imparts no intrinsic biological insights other than the important clue as to where to look in the genome for the abnormal gene. Third, strategies such as positional cloning or the positional candidate approach are used to identify the aberrant gene and its specific mutations within the linked region (Collins, 1995).

Linkage studies in disorders with complex inheritance have been bedevilled by negative results and by failure to replicate positive findings. A sense of frustration permeates current literature in the genetics of complex disorders. Carefully performed, large scale studies involving hundreds of sibpairs in disorders including multiple sclerosis and diabetes have been essentially negative (Bell and Lathrop, 1996; Lernmark and Ott, 1998). An emerging view is that such disorders are due to the summation of many genes of small effect and that identification of these genes may only be possible with very large-scale association studies. Such studies on a genome-wide basis are currently impossible due to incomplete marker sets and computational limitations.

The idiopathic generalized epilepsies (IGE) are the most common group of inherited human epilepsy and do not have simple inheritance. Like other complex disorders, linkage studies in IGE have generated controversial and conflicting claims. Previous authors have suggested the possibility of multifactorial, polygenic, oligogenic or two locus models for the disease (Andermann, 1982; Doose

and Baier, 1989; Greenberg et al., 1988a; 1992; Janz et al., 1992).

Two broad groups of IGE are now known - the classical idiopathic generalized epilepsies (Commission on Classification and Terminology of the International League Against Epilepsy, 1989) and the newly recognized genetic syndrome of generalized epilepsy with febrile seizures plus (GEFS<sup>+</sup>) (Scheffer and Berkovic, 1997; Singh et al., 1999).

The classical IGEs are divided into a number of clinically recognizable but overlapping sub-syndromes including childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy etc (Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Roger et al., 1992). The sub-syndromes are identified by age of onset and the pattern of seizure types (absence, myoclonus and tonic-clonic). Some patients, particularly those with tonic-clonic seizures alone do not fit a specifically recognized sub-syndrome. Arguments for regarding these as separate syndromes, yet recognizing that they are part of a neurobiological continuum, have been presented previously (Berkovic et al. 1987; 1994; Reutens and Berkovic, 1995).

GEFS<sup>+</sup> was originally recognized through large multi-generation families and comprises a variety of sub-syndromes. Febrile seizures plus (FS<sup>+</sup>) is a sub-syndrome where children have febrile seizures occurring outside the age range of 3 months to 6 years, or have associated febrile tonic-clonic seizures. Many family members have a phenotype indistinguishable from the classical febrile convulsion syndrome and some have FS<sup>+</sup> with additional absence, myoclonic, atonic, or complex partial seizures. The severe end of the GEFS<sup>+</sup> spectrum includes myoclonic-astatic epilepsy.

The cumulative incidence for epilepsy by age 30 years (proportion suffering from epilepsy at some time) is 1.5% (Hauser et al., 1993). Accurate estimates for the

cumulative incidence of the IGEs are unavailable. In epidemiological studies where attempts are made to subclassify epilepsies, rather few cases of IGE are diagnosed, and many cases are unclassified. This is probably because cases are rarely directly examined by epileptologists. In clinic- or office-based series seen by experts, most cases are classifiable and IGEs account for about 25% of cases. This suggests that about 0.3% of the population suffer from IGE at some time.

10 In outbred populations many patients with classical IGE appear to be sporadic as siblings and parents are usually unaffected. Systematic EEG studies on clinically unaffected family members show an increase in age-dependent occurrence of generalized epileptiform discharges compared to controls. In addition, to the approximate 0.3% of the population with clinical IGE, systematic EEG studies suggest that about 1% of healthy children have generalized epileptiform discharges while awake (Cavazzuti et al., 1980; Okubo et al., 1994).

20 Approximately 5-10% of first degree relatives of classical IGE probands have seizures with affected relatives usually having IGE phenotypes or febrile seizures. While nuclear families with 2-4 affected individuals are well recognized and 3 generation families or grandparent-grandchild pairs are occasionally observed (Italian League Against Epilepsy Genetic Collaborative Group, 1993), families with multiple affected individuals extending over 4 or more generations are exceptionally rare.

30 For GEFS<sup>+</sup>, however, a number of large multi-generation families showing autosomal dominant inheritance with incomplete penetrance are known. Similar to classical IGE, analysis of sporadic cases and small families with GEFS<sup>+</sup> phenotypes does not suggest simple Mendelian inheritance. Indeed, bilineal inheritance, where there is a history of epilepsy on maternal and paternal sides, is observed in both GEFS<sup>+</sup> and classical IGE families (Singh et al., 1999;

Italian League Against Epilepsy Genetic Collaborative Group, 1993).

Within single families with classical IGE or GEFS<sup>+</sup>, affected individuals often have different sub-syndromes. 5 The closer an affected relative is to the proband, the more similar are their sub-syndromes, and siblings often have similar sub-syndromes (Italian League Against Epilepsy Genetic Collaborative Group, 1993). Less commonly, families are observed where most, or all, known 10 affected individuals have one classical IGE sub-syndrome such as childhood absence epilepsy or juvenile myoclonic epilepsy (Italian League Against Epilepsy Genetic Collaborative Group, 1993).

Importantly, sub-syndromes are identical in affected 15 monozygous twins with IGE. In contrast, affected dizygous twins, may have the same or different sub-syndromes. Classical IGE and GEFS<sup>+</sup> sub-syndromes tend to segregate separately (Singh et al., 1999).

In some inbred communities, pedigree analysis 20 strongly suggests recessive inheritance for juvenile myoclonic epilepsy and other forms of IGE (Panayiotopoulos and Obeid, 1989; Berkovic et al., 2000). In such families, sub-syndromes are much more similar in affected siblings than in affected sib-pairs from outbred families. 25 Recently, a family with an infantile form of IGE with autosomal recessive inheritance, confirmed by linkage analysis, was described in Italy (Zara et al., 2000).

Most work on the molecular genetics of classical IGEs has been done on the sub-syndrome of juvenile myoclonic 30 epilepsy where a locus in proximity or within the HLA region on chromosome 6p was first reported in 1988 (Greenberg et al., 1988b). This finding was supported by two collaborating laboratories, in separate patient samples, and subsequently three groups provided further 35 evidence for a 6p locus for juvenile myoclonic epilepsy in some, but not all, of their families. However, genetic defects have not been found and the exact locus of the

gene or genes, in relationship to the HLA region, remains controversial. Strong evidence for linkage to chromosome 6 also comes from a study of a single large family with juvenile myoclonic epilepsy, but in this pedigree the locus is well outside the HLA region. A locus on chromosome 15q has also been suggested for juvenile myoclonic epilepsy, but was not confirmed by two other studies.

In general, the results of studies of the putative chromosomal 6p locus in the HLA region in patients with absence epilepsies or other forms of idiopathic generalized epilepsies have been negative. The major exception is that study of probands with tonic-clonic seizures on awakening, a sub-syndrome closely related to juvenile myoclonic epilepsy, suggests linkage to 6p.

Linkage for classical remitting childhood absence epilepsy remains elusive, but in a family with persisting absence evolving into a juvenile myoclonic epilepsy phenotype, linkage to chromosome 1p has been claimed. An Indian pedigree with persisting absence and tonic-clonic seizures may link to 8q24. Linkage to this region was also suggested by a non-parametric analysis in IGE, irrespective of subsyndrome, but was not confirmed in another study. Other loci for IGEs that have been reported in single studies include 3p14, 8p, 18 and possibly 5p. The unusual example of recessively inherited infantile onset IGE described in Italy maps to 16p in a single family.

Thus, like most disorders with complex inheritance, the literature on genetics of classical IGEs is confusing and contradictory. Some, and perhaps much, of this confusion is due to heterogeneity, with the likelihood of a number of loci for IGEs. The studies reviewed above were principally performed on multiple small families, so heterogeneity within and between samples is probable. Whether all, some, or none of the linkages reported above will be found to harbour relevant genes for IGE remains to

be determined. Most of the studies reviewed above used analysis methods assuming Mendelian inheritance, an assumption that is not correct for outbred communities. Some studies used multiple models (autosomal recessive, 5 autosomal dominant). Although parametric linkage analysis may be reliable in some circumstance of analyzing complex disease, it can lead to spurious findings as highlighted by the literature on linkage in major psychoses (Risch and Botstein, 1996).

10 In so far as GEFS<sup>+</sup> is concerned, linkage analysis on rare multi-generation large families with clinical evidence of a major autosomal dominant gene have demonstrated loci on chromosomes 19q and 2q. Both the 19q and 2q GEFS<sup>+</sup> loci have been confirmed in independently 15 ascertained large families, and genetic defects have been identified. Families linked to 19q are known and a mutation in the gene for the  $\beta 1$  subunit of the neuronal sodium channel (SCN1B) has been identified (Wallace et al., 1998). This mutation results in the loss of a 20 critical disulphide bridge of this regulatory subunit and causes a loss of function in vitro. Families linked to 2q are also known and mutations in the pore-forming  $\alpha$  subunit of the neuronal sodium channel (SCN1A) have been identified (PCT/AU01/01648; Wallace et al., 2001b; Escayg 25 et al., 2000). Studies on the more common small families with GEFS<sup>+</sup> have not revealed these or other mutations to date.

In addition to the SCN1B and SCN1A mutations in GEFS<sup>+</sup>, four other gene defects have been discovered for human 30 idiopathic epilepsies through the study of large families. Mutations in the alpha-4 subunit of the neuronal nicotinic acetylcholine receptor (CHRNA4) occur in the focal epilepsy syndrome of autosomal dominant nocturnal frontal lobe epilepsy (Australian patent AU-B-56247/96; Steinlein 35 et al., 1995). Mutations in the gamma-2 subunit of the GABA<sub>A</sub> receptor (GABRG2) have been identified in childhood absence epilepsy, febrile seizures (including febrile

seizures plus) and myoclonic epilepsy (PCT/AU01/00729; Wallace et al., 2001a). Finally, mutations in two potassium channel genes (KCNQ2 and KCNQ3) were identified in benign familial neonatal convulsions (Singh et al., 1998; Biervert et al., 1998; Charlier et al., 1998). Although initially regarded as a special form of IGE, this unusual syndrome is probably a form of inherited focal epilepsy.

Further to these studies, mutations in other genes have been identified to be causative of epilepsy. These include mutations in the beta-2 subunit (CHRNA2) of the neuronal nicotinic acetylcholine receptor (PCT/AU01/00541; Phillips et al., 2001) and the delta subunit (GABRD) of the GABA<sub>A</sub> receptor (PCT/AU01/00729).

A number of mouse models approximating human IGE are known. These mice mutants have ataxia in addition to generalized spike-and-wave discharges with absences or tonic-clonic seizures. Recessive mutations in calcium channel subunit genes have been found in lethargic (CACNB4), tottering/leaner (CACNA1A), and stargazer (CACNG2) mutants. The slow-wave epilepsy mouse mutant has a mutation in the sodium/hydrogen exchanger gene, which may have important downstream effects on pH-sensitive ion channels.

The human and mouse literature is now suggesting that the idiopathic epilepsies comprise a family of channelopathies with mutations in ion channel subunits of voltage-gated (eg SCN1A, SCN1B, KCNQ2, KCNQ3) or ligand-gated (eg CHRNA4, CHRNA2, GABRG2, GABRD) types. These channels are typically comprised of a number of subunits, specified by genes on different chromosomes. The stoichiometry and conformation of ion channel subunits are not yet well understood, but many have multiple subunits in a variety of combinations.

The involvement of ion channels in other neuro/physiological disorders has also been observed (reviewed in Dworakowska and Dolowy, 2000). Mutations in

voltage-gated sodium, potassium, calcium and chloride channels as well as ligand-gated channels such as the acetylcholine and GABA receptors may lead to physiological disorders such as hyper- and hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia and cardiac arrhythmias. Neurological disorders other than epilepsy that are associated with ion channel mutations include episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, as well as neuropathic pain, inflammatory pain and chronic/acute pain. Some kidney disorders such as Bartter's syndrome, polycystic kidney disease and Dent's disease, secretion disorders such as hyperinsulinemic hypoglycemia of infancy and cystic fibrosis, and vision disorders such as congenital stationary night blindness and total colour-blindness may also be linked to mutations in ion channels.

#### Disclosure of the Invention

In a new genetic model for the idiopathic generalised epilepsies (IGEs) described in PCT/AU01/00872 (the disclosure of which is incorporated herein by reference) it has been postulated that most classical IGE and GEFS<sup>+</sup> cases are due to the combination of two mutations in multi-subunit ion channels. These are typically point mutations resulting in a subtle change of function. The critical postulate is that two mutations, usually, but not exclusively, in different subunit alleles ("digenic model"), are required for clinical expression of IGE. It was further proposed that

- a) A number of different mutated subunit pairs can be responsible for IGE. Combinations of two mutated subunits lead to an IGE genotype with ~30% penetrance.
- b) The total allele frequency of mutated subunits is ~8%. It was calculated that approximately 15% of the population has one or more mutated



subunit genes and 1% have two or more mutated subunits.

- 5 c) Sub-syndromes are principally determined by the specific combination of mutated subunit pairs, although one or more other genes, including ion channel subunits, of smaller effect may modify the phenotype.
- 10 d) Mutated subunit combinations that cause classical IGEs are largely separate from those that cause GEFS<sup>+</sup>, although some subunits may be involved in both syndromes.
- 15 e) Individuals with single 'change of function' mutations would not have IGE, but such mutations may contribute to simple febrile seizures, which are observed with increased frequency in relatives of IGE probands.

The model also proposes that subunit mutations with more severe functional consequences (eg breaking a disulphide bridge in SCN1B or amino acid substitution in the pore forming regions of SCN1A for GEFS<sup>+</sup>) cause autosomal dominant generalized epilepsies with a penetrance of 60-90%. The precise sub-syndromes in GEFS<sup>+</sup> are determined by minor allelic variation or mutations in other ion channel subunits. Such "severe" mutations are rare (allele frequency <0.01%) and are infrequent causes of GEFS<sup>+</sup>. They very rarely, or perhaps never, cause classical IGE.

30 The identification of molecular changes in ion channel subunits is therefore a significant step towards the elucidation of genetic variants that alone or in combination (based on the digenic model) give rise to an epilepsy phenotype, and to other neuro/physiological disorders associated with ion channel dysfunction.

35 The present inventors have identified a number of novel mutations or variants in genes encoding subunits of ion channels in individuals with epilepsy. It will be appreciated that for each molecular defect one can provide

an isolated nucleic acid molecule coding for a protein having a biological function as part of an ion channel in a mammal, wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred so as to affect the functioning of the ion channel. In some instances this single mutation alone will produce a phenotype of epilepsy or other neuro/physiological disorders associated with ion channel dysfunction.

10 In the case where a single mutation alone does not produce, say, an epilepsy phenotype, there would be provided one or more additional isolated nucleic acid molecules coding for proteins having a biological function as part of an ion channel in a mammal, wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred so as to affect the functioning of the ion channel. The cumulative effect of the mutations in each isolated nucleic acid molecule in vivo is to produce a epilepsy or another neuro/physiological disorders in said mammal. The mutations may be in nucleic acid molecules coding for protein subunits belonging to the same ion channel or may be in nucleic acid molecules coding for protein subunits that belong to different ion channels.

25 Typically such mutations are point mutations and the ion channels are voltage-gated channels such as a sodium, potassium, calcium or chloride channels or are ligand-gated channels such as members of the nAChR/GABA super family of receptors, or a functional fragment or homologue thereof.

30 Mutations may include those in non-coding regions of the ion channel subunits (eg mutations in the promoter region which affect the level of expression of the subunit gene, mutations in intronic sequences which affect the correct splicing of the subunit during mRNA processing, or mutations in the 5' or 3' untranslated regions that can affect translation or stability of the mRNA). Mutations

may also and more preferably will be in coding regions of the ion channel subunits (eg nucleotide mutations may give rise to an amino acid change in the encoded protein or nucleotide mutations that do not give rise to an amino acid change but may affect the stability of the mRNA).

Mutation combinations may be selected from, but are not restricted to, those identified in Table 1.

Accordingly in one aspect of the present invention there is provided a method of identifying a subject predisposed to a disorder associated with ion channel dysfunction, comprising ascertaining whether at least one of the genes encoding ion channel subunits in said subject has undergone a mutation event as set forth in one of SEQ ID Numbers: 1-62.

In another aspect of the present invention there is provided an isolated nucleic acid molecule encoding a mutant or variant ion channel subunit wherein a mutation event has occurred as set forth in one of SEQ ID Numbers: 1-62.

The mutation event disrupts the functioning of an ion channel so as to produce a phenotype of epilepsy, and/or one or more other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness and total colour-blindness, either alone or in combination with one or more additional mutations or variations in the ion channel subunit genes.

In another aspect of the present invention there is provided an isolated nucleic acid molecule encoding a

mutant KCNQ2 subunit, wherein the mutation event has occurred in the C-terminal domain of the KCNQ2 subunit and leads to a disturbance in the calmodulin binding affinity of the subunit, so as to produce an epilepsy phenotype.

5 In one form of the invention, the mutations are in exon 8 or exon 15 of the KCNQ2 subunit and result in the replacement of an arginine residue with a glycine residue at amino acid position 353, or the replacement of a  
10 leucine residue with an arginine at amino acid position 619. The R353G mutation occurs as a result of a C to G nucleotide substitution at position 1057 of the KCNQ2 coding sequence as shown in SEQ ID NO: 34. The L619R mutation occurs as a result of a T to G nucleotide substitution at position 1856 of the KCNQ2 coding sequence  
15 as shown in SEQ ID NO: 37.

In a further form of the invention, the mutations are in exon 11 or exon 14 of the KCNQ2 subunit and result in the replacement of an arginine residue with a stop codon at amino acid position 430, or the replacement of an  
20 arginine residue with a serine at amino acid position 570. The R430X mutation occurs as a result of a C to T nucleotide substitution at position 1288 of the KCNQ2 coding sequence as shown in SEQ ID NO: 35. The R570S mutation occurs as a result of an A to T nucleotide  
25 substitution at position 1710 of the KCNQ2 coding sequence as shown in SEQ ID NO: 36.

Typically these mutations create a phenotype of benign familial neonatal seizures (BFNS).

In a further aspect of the present invention there is  
30 provided a combination of two or more isolated nucleic acid molecules each having a novel mutation event as laid out in Table 1. The cumulative effect of the mutations in each isolated nucleic acid molecule *in vivo* is to produce an epilepsy or another disorder associated with ion  
35 channel dysfunction as described above in said mammal.

In a particularly preferred embodiment of the present invention, the isolated nucleic acid molecules have a

nucleotide sequence as shown in any one of SEQ ID Numbers: 1-62. The sequences correspond to the novel DNA mutations or variants laid out in Table 1.

5 In another aspect of the present invention there is provided an isolated nucleic acid molecule comprising any one of the nucleotide sequences set forth in SEQ ID Numbers: 1-62.

10 In another aspect of the present invention there is provided an isolated nucleic acid molecule consisting of any one of the nucleotide sequences set forth in SEQ ID Numbers: 1-62.

The nucleotide sequences of the present invention can be engineered using methods accepted in the art for a variety of purposes. These include, but are not limited to, modification of the cloning, processing, and/or expression of the gene product. PCR reassembly of gene fragments and the use of synthetic oligonucleotides allow the engineering of the nucleotide sequences of the present invention. For example, oligonucleotide-mediated site-directed mutagenesis can introduce further mutations that create new restriction sites, alter expression patterns and produce splice variants etc.

20 As a result of the degeneracy of the genetic code, a number of polynucleotide sequences, some that may have minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention includes each and every possible variation of a polynucleotide sequence that could be made by selecting combinations based on possible codon choices.

30 These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequences of the present invention, and all such variations are to be considered as being specifically disclosed.

35 The nucleic acid molecules of this invention are typically DNA molecules, and include cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and

antisense strands, and may be chemically or biochemically modified, or may contain non-natural or derivatised nucleotide bases as will be appreciated by those skilled in the art. Such modifications include labels, 5 methylation, intercalators, alkylators and modified linkages. In some instances it may be advantageous to produce nucleotide sequences possessing a substantially different codon usage than that of the polynucleotide sequences of the present invention. For example, codons 10 may be selected to increase the rate of expression of the peptide in a particular prokaryotic or eukaryotic host corresponding with the frequency that particular codons are utilized by the host. Other reasons to alter the nucleotide sequence without altering the encoded amino 15 acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring mutated sequence.

The invention also encompasses production of nucleic 20 acid sequences of the present invention entirely by synthetic chemistry. Synthetic sequences may be inserted into expression vectors and cell systems that contain the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable 25 host. These elements may include regulatory sequences, promoters, 5' and 3' untranslated regions and specific initiation signals (such as an ATG initiation codon and Kozak consensus sequence) which allow more efficient translation of sequences encoding the polypeptides of the 30 present invention. In cases where the complete coding sequence, including the initiation codon and upstream regulatory sequences, are inserted into the appropriate expression vector, additional control signals may not be needed. However, in cases where only coding sequence, or a 35 fragment thereof, is inserted, exogenous translational control signals as described above should be provided by the vector. Such signals may be of various origins, both

natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used (Scharf et al., 1994).

5 The invention also includes nucleic acid molecules that are the complements of the sequences described herein.

10 The present invention allows for the preparation of purified polypeptide or protein from the polynucleotides of the present invention, or variants thereof. In order to do this, host cells may be transformed with a novel nucleic acid molecule as described above, or with nucleic acid molecules encoding two or more mutant ion channel subunits. If the mutant subunits form a part of the same ion channel a receptor protein containing two or more  
15 mutant subunits may be isolated. If the mutant subunits are subunits of different ion channels the host cells will express two or more mutant receptor proteins. Typically said host cells are transfected with an expression vector comprising a DNA molecule according to the invention or,  
20 in particular, DNA molecules encoding two or more mutant ion channel subunits. A variety of expression vector/host systems may be utilized to contain and express sequences encoding polypeptides of the invention. These include, but are not limited to, microorganisms such as bacteria transformed with plasmid or cosmid DNA expression vectors;  
25 yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); or mouse or other animal or human tissue cell systems. Mammalian cells can also be used to express  
30 a protein using a vaccinia virus expression system. The invention is not limited by the host cell or vector employed.

35 The polynucleotide sequences, or variants thereof, of the present invention can be stably expressed in cell lines to allow long term production of recombinant proteins in mammalian systems. Sequences encoding the polypeptides of the present invention can be transformed

into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. The selectable marker confers resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode a protein may be designed to contain signal sequences which direct secretion of the protein through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, glycosylation, phosphorylation, and acylation. Post-translational cleavage of a "prepro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells having specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO or HeLa cells), are available from the American Type Culture Collection (ATCC) and may be chosen to ensure the correct modification and processing of the foreign protein.

When large quantities of the protein product of the gene are needed, such as for antibody production, vectors which direct high levels of expression of this protein may be used, such as those containing the T5 or T7 inducible bacteriophage promoter. The present invention also includes the use of the expression systems described above in generating and isolating fusion proteins which contain



important functional domains of the protein. These fusion proteins are used for binding, structural and functional studies as well as for the generation of appropriate antibodies.

5        In order to express and purify the protein as a fusion protein, the appropriate cDNA sequence is inserted into a vector which contains a nucleotide sequence encoding another peptide (for example, glutathionine succinyl transferase). The fusion protein is expressed and  
10       recovered from prokaryotic or eukaryotic cells. The fusion protein can then be purified by affinity chromatography based upon the fusion vector sequence. The desired protein is then obtained by enzymatic cleavage of the fusion protein.

15       Fragments of the polypeptides of the present invention may also be produced by direct peptide synthesis using solid-phase techniques. Automated synthesis may be achieved by using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Various fragments of this protein may be  
20       synthesized separately and then combined to produce the full-length molecule.

      The present invention is also concerned with polypeptides having a biological function as an ion channel in a mammal, wherein a mutation event selected  
25       from the group consisting of substitutions, deletions, truncations, insertions and rearrangements has occurred so as to affect the functioning of the ion channel. In some instances this single mutation alone will produce an epilepsy phenotype or other neuro/physiological disorders  
30       associated with ion channel dysfunction.

      In the case where a single mutation alone does not produce, say, an epilepsy phenotype, there would be provided one or more additional isolated mammalian polypeptides having biological functions as part of an ion  
35       channel in a mammal, wherein a mutation event selected from the group consisting of substitutions, deletions, truncations, insertions and rearrangements has occurred so

as to affect the functioning of the ion channel. The cumulative effect of the mutations in each isolated mammalian polypeptide *in vivo* being to produce epilepsy or another neuro/physiological disorders in said mammal. The mutations may be in polypeptide subunits belonging to the same ion channel as described above, but may also be in polypeptide subunits that belong to different ion channels.

Typically the mutation is an amino acid substitution and the ion channel is a voltage-gated channel such as a sodium, potassium, calcium or chloride channel or a ligand-gated channel such as a member of the nAChR/GABA super family of receptors, or a functional fragment or homologue thereof.

Mutation combinations may be selected from, but are not restricted to, those represented in Table 1.

Accordingly, in a further aspect of the present invention there is provided an isolated polypeptide, said polypeptide being a mutant or variant ion channel subunit wherein a mutation event has occurred such that the polypeptide has the amino acid sequence set forth in one of SEQ ID Numbers: 63-76. The mutation event disrupts the functioning of an ion channel so as to produce a phenotype of epilepsy, and/or one or more other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness and total colour-blindness.

In a particularly preferred embodiment of the present invention, the isolated polypeptide has an amino acid

sequence as shown in any one of SEQ ID Numbers: 63-76. The sequences correspond to the novel amino acid changes laid out in Table 1 for those instances where the DNA mutation results in an amino acid change.

5       According to still another aspect of the present invention there is provided an isolated polypeptide, said polypeptide being a mutant KCNQ2 subunit, wherein the mutation event has occurred in the C-terminal domain of the KCNQ2 subunit and leads to a disturbance in the  
10       calmodulin binding affinity of the subunit, so as to produce an epilepsy phenotype.

      In one form of the invention the mutations are substitutions in which an arginine residue is replaced with a glycine residue, or a leucine residue is replaced  
15       with an arginine. Preferably the substitutions are R353G and L619R transitions as illustrated by SEQ ID NOS: 73 and 76 respectively.

      In a further form of the invention the mutations result in the replacement of an arginine for a stop codon,  
20       or an arginine is replaced with a serine. Preferably the mutations are R430X and R570S transitions as illustrated by SEQ ID NOS: 74 and 75 respectively.

      In a still further aspect of the present invention there is provided a combination of two or more isolated  
25       polypeptides each having a novel mutation event as laid out in Table 1. The cumulative effect of the mutations in each isolated polypeptide molecule in vivo is to produce an epilepsy or another disorder associated with ion channel dysfunction as described above in said mammal.

30       In a particularly preferred embodiment of the present invention, the isolated polypeptides have an amino acid sequence as shown in any one of SEQ ID Numbers: 63-76. The sequences correspond to the novel amino acid changes laid out in Table 1.

35       According to still another aspect of the present invention there is provided an isolated polypeptide

comprising the amino acid sequence set forth in any one of SEQ ID Numbers: 63-76.

According to still another aspect of the present invention there is provided a polypeptide consisting of the amino acid sequence set forth in any one of SEQ ID Numbers: 63-76.

According to still another aspect of the present invention there is provided a method of preparing a polypeptide, comprising the steps of:

- (1) culturing host cells transfected with an expression vector comprising a nucleic acid molecule as described above under conditions effective for polypeptide production; and
- (2) harvesting the mutant ion channel subunit.

The mutant ion channel subunit may be allowed to assemble with other subunits constituting the channel that are either wild-type or themselves mutant subunits, whereby the assembled ion channel is harvested.

According to still another aspect of the invention there is provided a polypeptide which is the product of the process described above.

Substantially purified protein or fragments thereof can then be used in further biochemical analyses to establish secondary and tertiary structure. Such methodology is known in the art and includes, but is not restricted to, X-ray crystallography of crystals of the proteins or of the assembled ion channel incorporating the proteins or by nuclear magnetic resonance (NMR). Determination of structure allows for the rational design of pharmaceuticals to interact with the ion channel as a whole or through interaction with a specific subunit protein (see drug screening below), alter the overall ion channel protein charge configuration or charge interaction with other proteins, or to alter its function in the cell.

It will be appreciated that the mutant ion channel subunits included as part of the present invention will be useful in further applications which include a variety of

hybridisation and immunological assays to screen for and detect the presence of either a normal or mutated gene or gene product. The invention enables therapeutic methods for the treatment of epilepsy as well as other disorders associated with ion channel dysfunction and also enables methods for the diagnosis of epilepsy as well as other disorders associated with ion channel dysfunction.

#### Therapeutic Applications

According to still another aspect of the invention there is provided a method of treating epilepsy as well as other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness, comprising administering a selective antagonist, agonist or modulator of an ion channel or ion channel subunit, when the ion channel contains a mutation in a subunit comprising the channel, as described above, to a subject in need of such treatment. Said mutation event may be causative of the disorder when expressed alone or when expressed in combination with one or more additional mutations in subunits of the same or different ion channels, which are typically those identified in Table 1.

In still another aspect of the invention there is provided the use of a selective antagonist, agonist or modulator of an ion channel or ion channel subunit when the ion channel contains a mutation in a subunit comprising the channel, as described above, said mutation being causative of epilepsy as well as other disorders

associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness, when expressed alone or when expressed in combination with a second mutation in a subunit of the same or different ion channel, as described above, in the manufacture of a medicament for the treatment of the disorder.

In one aspect, a suitable antagonist, agonist or modulator will restore wild-type function to the ion channel or channels containing the mutations of the present invention, or will negate the effects the mutant channel or channels have on cell function.

Using methods well known in the art, a mutant ion channel may be used to produce antibodies specific for the mutant channel that is causative of the disease or to screen libraries of pharmaceutical agents to identify those that bind the mutant ion channel.

In one aspect, an antibody, which specifically binds to a mutant ion channel or mutant ion channel subunit of the invention, may be used directly as an agonist, antagonist or modulator, or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues that express the mutant ion channel.

In a still further aspect of the invention there is provided an antibody which is immunologically reactive with a polypeptide as described above, but not with a wild-type ion channel or ion channel subunit thereof.

In particular, there is provided an antibody to an assembled ion channel containing a mutation in a subunit

comprising the receptor, which is causative of epilepsy or another disorder associated with ion channel dysfunction when expressed alone or when expressed in combination with one or more other mutations in subunits of the same or different ion channels. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies as would be understood by the person skilled in the art.

For the production of antibodies, various hosts including rabbits, rats, goats, mice, humans, and others may be immunized by injection with a polypeptide as described above or with any fragment or oligopeptide thereof which has immunogenic properties. Various adjuvants may be used to increase immunological response and include, but are not limited to, Freund's, mineral gels such as aluminium hydroxide, and surface-active substances such as lysolecithin. Adjuvants used in humans include BCG (bacilli Calmette-Guerin) and Corynebacterium parvum.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to the mutant ion channel have an amino acid sequence consisting of at least 5 amino acids, and, more preferably, of at least 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of ion channel amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to a mutant ion channel may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (For example, see Kohler

et al., 1975; Kozbor et al., 1985; Cote et al., 1983; Cole et al., 1984).

Monoclonal antibodies produced may include, but are not limited to, mouse-derived antibodies, humanised  
5 antibodies and fully human antibodies.

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (For  
10 example, see Orlandi et al., 1989; Winter and Milstein, 1991).

Antibody fragments which contain specific binding sites for a mutant ion channel may also be generated. For example, such fragments include, F(ab')<sub>2</sub> fragments  
15 produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the  
20 desired specificity. (For example, see Huse et al., 1989).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or  
25 monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between an ion channel and its specific antibody. A two-site, monoclonal-based immunoassay utilizing antibodies reactive to two  
30 non-interfering ion channel epitopes is preferred, but a competitive binding assay may also be employed.

In a further aspect of the invention there is provided a method of treating epilepsy as well as other disorders associated with ion channel dysfunction,  
35 including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia,



migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness, comprising administering an isolated nucleic acid molecule which is the complement (antisense) of any one of the nucleic acid molecules described above and which encodes an RNA molecule that hybridizes with the mRNA encoding a mutant ion channel subunit of the invention, to a subject in need of such treatment.

In a still further aspect of the invention there is provided the use of an isolated nucleic acid molecule which is the complement (antisense) of a nucleic acid molecule of the invention and which encodes an RNA molecule that hybridizes with the mRNA encoding a mutant ion channel subunit of the invention, in the manufacture of a medicament for the treatment of epilepsy as well as other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

Typically, a vector expressing the complement (antisense) of the polynucleotides of the invention may be administered to a subject in need of such treatment. Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be

introduced into stem cells taken from the patient and  
clonally propagated for autologous transplant back into  
that same patient. Delivery by transfection, by liposome  
injections, or by polycationic amino polymers may be  
5 achieved using methods which are well known in the art.  
(For example, see Goldman et al., 1997).

Additional antisense or gene-targeted silencing  
strategies may include, but are not limited to, the use of  
antisense oligonucleotides, injection of antisense RNA,  
10 transfection of antisense RNA expression vectors, and the  
use of RNA interference (RNAi) or short interfering RNAs  
(siRNA). Still further, catalytic nucleic acid molecules  
such as DNazymes and ribozymes may be used for gene  
silencing (Breaker and Joyce, 1994; Haseloff and Gerlach,  
15 1988). These molecules function by cleaving their target  
mRNA molecule rather than merely binding to it as in  
traditional antisense approaches.

In a further aspect, a suitable agonist, antagonist  
or modulator may include peptides, phosphopeptides or  
20 small organic or inorganic compounds that can restore  
wild-type activity of ion channels containing mutations in  
the subunits which comprise the channels as described  
above.

Peptides, phosphopeptides or small organic or  
25 inorganic compounds suitable for therapeutic applications  
may be identified using nucleic acids and peptides of the  
invention in drug screening applications as described  
below. Molecules identified from these screens may also be  
of therapeutic application in affected individuals  
30 carrying other ion channel subunit gene mutations if the  
molecule is able to correct the common underlying  
functional deficit imposed by these mutations and those of  
the invention.

There is therefore provided a method of treating  
35 epilepsy as well as other disorders associated with ion  
channel dysfunction comprising administering a compound  
that is a suitable agonist, antagonist or modulator of an

ion channel and that has been identified using the mutant ion channel subunits of the invention.

5 In some instances, an appropriate approach for treatment may be combination therapy. This may involve the administering an antibody or complement (antisense) to a mutant ion channel or ion channel subunit of the invention to inhibit its functional effect, combined with administration of wild-type ion channel subunits which may restore levels of wild-type ion channel formation to normal levels. Wild-type ion channel subunits of the invention can be administered using gene therapy approaches as described above for complement administration.

15 There is therefore provided a method of treating epilepsy as well as other disorders associated with ion channel dysfunction comprising administration of an antibody or complement to a mutant ion channel or ion channel subunit of the invention in combination with administration of wild-type ion channel subunits.

20 In still another aspect of the invention there is provided the use of an antibody or complement to a mutant ion channel or ion channel subunit of the invention in combination with the use of wild-type ion channel subunits, in the manufacture of a medicament for the treatment of epilepsy as well as other disorders associated with ion channel dysfunction.

25 In further embodiments, any of the agonists, antagonists, modulators, antibodies, complementary sequences or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents may be made by those skilled in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, therapeutic efficacy with lower dosages of

each agent may be possible, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

#### Drug Screening

According to still another aspect of the invention, nucleic acid molecules of the invention as well as peptides of the invention, particularly purified mutant ion channel subunit polypeptide and cells expressing these, are useful for the screening of candidate pharmaceutical agents for the treatment of epilepsy as well as other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

Still further, it provides the use of a polypeptide complex for the screening of candidate pharmaceutical compounds.

Still further, it provides the use wherein high throughput screening techniques are employed.

Compounds that can be screened in accordance with the invention include, but are not limited to peptides (such as soluble peptides), phosphopeptides and small organic or inorganic molecules (such as natural product or synthetic chemical libraries and peptidomimetics).

In one embodiment, a screening assay may include a cell-based assay utilising eukaryotic or prokaryotic host cells that are stably transformed with recombinant molecules expressing the polypeptides or fragments of the invention, in competitive binding assays. Binding assays will measure the formation of complexes between a specific mutant ion channel subunit polypeptide or ion channel incorporating a mutant ion channel subunit polypeptide, and the compound being tested, or will measure the degree to which a compound being tested will inhibit or restore the formation of a complex between a specific mutant ion channel subunit polypeptide or ion channel incorporating a mutant ion channel subunit polypeptide, and its interactor or ligand.

The invention is particularly useful for screening compounds by using the polypeptides of the invention in transformed cells, transfected or injected oocytes, or animal models bearing mutated ion channel subunits such as transgenic animals or gene targeted (knock-in) animals (see transformed hosts). Drug candidates can be added to cultured cells that express a single mutant ion channel subunit or combination of mutant ion channel subunits (appropriate wild-type ion channel subunits should also be expressed for receptor assembly), can be added to oocytes transfected or injected with either a mutant ion channel subunit or combination of mutant ion channel subunits (appropriate wild-type ion channel subunits must also be injected for receptor assembly), or can be administered to an animal model containing a mutant ion channel or combination of mutant ion channels. Determining the ability of the test compound to modulate mutant ion channel activity can be accomplished by a number of techniques known in the art. These include for example measuring the effect on the current of the channel (e.g. calcium-, chloride-, sodium-, potassium-ion flux) as compared to the current of a cell or animal containing wild-type ion channels. Current in cells can be measured

by a number of approaches including the patch-clamp technique (methods described in Hamill et al, 1981) or using fluorescence based assays as are known in the art (see Gonzalez et al. 1999). Drug candidates that alter the  
5 current to a more normal level are useful for treating or preventing epilepsy as well as other disorders associated with ion channel dysfunction.

Non cell-based assays may also be used for identifying compounds that can inhibit or restore binding  
10 between the polypeptides of the invention or ion channels incorporating the polypeptides of the invention, and their interactors. Such assays are known in the art and include for example AlphaScreen technology (PerkinElmer Life Sciences, MA, USA). This application relies on the use of  
15 beads such that each interaction partner is bound to a separate bead via an antibody. Interaction of each partner will bring the beads into proximity, such that laser excitation initiates a number of chemical reactions ultimately leading to fluorophores emitting a light  
20 signal. Candidate compounds that inhibit the binding of the mutant ion channel subunit, or ion channel incorporating the mutant subunit, with its interactor will result in loss of light emission, while candidate compounds that restore the binding of the mutant ion  
25 channel subunit, or ion channel incorporating the mutant subunit, with its interactor will result in positive light emission. These assays ultimately enable identification and isolation of the candidate compounds.

High-throughput drug screening techniques may also  
30 employ methods as described in WO84/03564. Small peptide test compounds synthesised on a solid substrate can be assayed for mutant ion channel subunit polypeptide or mutant ion channel binding. Bound mutant ion channel or mutant ion channel subunit polypeptide is then detected by  
35 methods well known in the art. In a variation of this technique, purified polypeptides of the invention can be

coated directly onto plates to identify interacting test compounds.

The invention also contemplates the use of competition drug screening assays in which neutralizing antibodies capable of specifically binding the mutant ion channel compete with a test compound for binding thereto. In this manner, the antibodies can be used to detect the presence of any peptide that shares one or more antigenic determinants of the mutant ion channel.

10 The polypeptides of the present invention may also be used for screening compounds developed as a result of combinatorial library technology. This provides a way to test a large number of different substances for their ability to modulate activity of a polypeptide. A substance  
15 identified as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many *in vivo* pharmaceutical applications. In addition, a mimic or mimetic of the substance may be designed for  
20 pharmaceutical use. The design of mimetics based on a known pharmaceutically active compound ("lead" compound) is a common approach to the development of novel pharmaceuticals. This is often desirable where the original active compound is difficult or expensive to  
25 synthesise or where it provides an unsuitable method of administration. In the design of a mimetic, particular parts of the original active compound that are important in determining the target property are identified. These parts or residues constituting the active region of the  
30 compound are known as its pharmacophore. Once found, the pharmacophore structure is modelled according to its physical properties using data from a range of sources including x-ray diffraction data and NMR. A template molecule is then selected onto which chemical groups which  
35 mimic the pharmacophore can be added. The selection can be made such that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, does not

degrade *in vivo* and retains the biological activity of the lead compound. Further optimisation or modification can be carried out to select one or more final mimetics useful for *in vivo* or clinical testing.

5        It is also possible to isolate a target-specific antibody and then solve its crystal structure. In principle, this approach yields a pharmacophore upon which subsequent drug design can be based as described above. It may be possible to avoid protein crystallography  
10 altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analogue of the original receptor. The anti-id could then be used to  
15 isolate peptides from chemically or biologically produced peptide banks.

Another alternative method for drug screening relies on structure-based rational drug design. Determination of the three dimensional structure of the polypeptides of the  
20 invention, or the three dimensional structure of the ion channels which incorporate these polypeptides allows for structure-based drug design to identify biologically active lead compounds.

Three dimensional structural models can be generated  
25 by a number of applications, some of which include experimental models such as x-ray crystallography and NMR and/or from *in silico* studies of structural databases such as the Protein Databank (PDB). In addition, three dimensional structural models can be determined using a  
30 number of known protein structure prediction techniques based on the primary sequences of the polypeptides (e.g. SYBYL - Tripos Associated, St. Louis, MO), *de novo* protein structure design programs (e.g. MODELER - MSI Inc., San Diego, CA, or MOE - Chemical Computing Group, Montreal,  
35 Canada) or *ab initio* methods (e.g. see US Patent Numbers 5331573 and 5579250).



Once the three dimensional structure of a polypeptide or polypeptide complex has been determined, structure-based drug discovery techniques can be employed to design biologically-active compounds based on these three dimensional structures. Such techniques are known in the art and include examples such as DOCK (University of California, San Francisco) or AUTODOCK (Scripps Research Institute, La Jolla, California). A computational docking protocol will identify the active site or sites that are deemed important for protein activity based on a predicted protein model. Molecular databases, such as the Available Chemicals Directory (ACD) are then screened for molecules that complement the protein model.

Using methods such as these, potential clinical drug candidates can be identified and computationally ranked in order to reduce the time and expense associated with typical 'wet lab' drug screening methodologies.

Compounds identified through screening procedures as described above, and which are based on the use of the mutant nucleic acid and polypeptides of the invention, can also be tested for their effect on correcting the functional deficit imposed by other gene mutations in affected individuals including other ion channel subunit mutations.

Such compounds form a part of the present invention, as do pharmaceutical compositions containing these and a pharmaceutically acceptable carrier.

#### Pharmaceutical Preparations

Compounds identified from screening assays and shown to restore ion channel wild-type activity can be administered to a patient at a therapeutically effective dose to treat or ameliorate epilepsy as well as other disorders associated with ion channel dysfunction, as described above. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of the disorder.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The data obtained from these studies can then be used in the formulation of  
5 a range of dosages for use in humans.

Pharmaceutical compositions for use in accordance with the present invention can be formulated in a conventional manner using one or more physiological acceptable carriers, excipients or stabilisers which are  
10 well known. Acceptable carriers, excipients or stabilizers are non-toxic at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues)  
15 polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; binding agents including hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates  
20 including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or non-ionic surfactants such as Tween, Pluronic or polyethylene glycol (PEG).

25 The formulation of pharmaceutical compositions for use in accordance with the present invention will be based on the proposed route of administration. Routes of administration may include, but are not limited to, inhalation, insufflation (either through the mouth or  
30 nose), oral, buccal, rectal or parental administration.

#### Diagnostic Applications

Polynucleotide sequences encoding an ion channel subunit may be used for the diagnosis of epilepsy, as well  
35 as other as other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant

hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness, and the use of the nucleic acid molecules incorporated as part of the invention in diagnosis of these disorders, or a predisposition to these disorders, is therefore contemplated. The nucleic acid molecules incorporating the novel mutation events laid out in Table 1 may be used for this purpose.

The polynucleotides that may be used for diagnostic purposes include oligonucleotide sequences, genomic DNA and complementary RNA and DNA molecules. The polynucleotides may be used to detect and quantitate gene expression in biological samples. Genomic DNA used for the diagnosis may be obtained from body cells, such as those present in the blood, tissue biopsy, surgical specimen, or autopsy material. The DNA may be isolated and used directly for detection of a specific sequence or may be amplified by the polymerase chain reaction (PCR) prior to analysis. Similarly, RNA or cDNA may also be used, with or without PCR amplification. To detect a specific nucleic acid sequence, hybridisation using specific oligonucleotides, restriction enzyme digest and mapping, PCR mapping, RNase protection, and various other methods may be employed. Oligonucleotides specific to particular sequences can be chemically synthesized and labelled radioactively or nonradioactively and hybridised to individual samples immobilized on membranes or other solid-supports or in solution. The presence, absence or excess expression of any one of the mutant ion channel genes of the invention may then be visualized using

methods such as autoradiography, fluorometry, or colorimetry.

5 In a further diagnostic approach, the nucleotide sequences of the invention may be useful in assays that detect the presence of associated disorders, particularly those mentioned previously. The nucleotide sequences may be labelled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridisation complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

20 In order to provide a basis for the diagnosis or prognosis of epilepsy and other disorders as described above, which are associated with the ion channel subunit mutations or variants of the invention, the nucleotide sequence of each gene can be compared between normal tissue and diseased tissue in order to establish whether the patient expresses a mutant gene.

30 In order to provide a basis for the diagnosis of a disorder associated with abnormal expression of an ion channel subunit gene of the invention, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding the relevant ion channel subunit gene, under conditions suitable for hybridisation or amplification. Standard hybridisation may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known

amount of a substantially purified polynucleotide is used. Another method to identify a normal or standard profile for expression of an ion channel subunit gene is through quantitative RT-PCR studies. RNA isolated from body cells of a normal individual is reverse transcribed and real-time PCR using oligonucleotides specific for the relevant gene is conducted to establish a normal level of expression of the gene. Standard values obtained in both these examples may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridisation assays or quantitative RT-PCR studies may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

According to a further aspect of the invention there is provided the use of a polypeptide as described above in the diagnosis of epilepsy as well as other disorders associated with ion channel dysfunction, including but not restricted to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness or total colour-blindness.

When a diagnostic assay is to be based upon proteins constituting an ion channel, a variety of approaches are possible. For example, diagnosis can be achieved by

monitoring differences in the electrophoretic mobility of normal and mutant proteins that form the ion channel. Such an approach will be particularly useful in identifying mutants in which charge substitutions are present, or in  
5 which insertions, deletions or substitutions have resulted in a significant change in the electrophoretic migration of the resultant protein. Alternatively, diagnosis may be based upon differences in the proteolytic cleavage patterns of normal and mutant proteins, differences in  
10 molar ratios of the various amino acid residues, or by functional assays demonstrating altered function of the gene products.

In another aspect, antibodies that specifically bind mutant ion channels may be used for the diagnosis of a  
15 disorder, or in assays to monitor patients being treated with a complete ion channel or agonists, antagonists, modulators or inhibitors of an ion channel. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic  
20 assays for ion channels include methods that utilize the antibody and a label to detect a mutant ion channel in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labelled by covalent or non-covalent attachment of  
25 a reporter molecule.

A variety of protocols for measuring the presence of mutant ion channels, including but not restricted to, ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing a disorder. The expression of a  
30 mutant ion channel or combination of mutant ion channels is established by combining body fluids or cell extracts taken from test mammalian subjects, preferably human, with antibody to the ion channel or channels under conditions suitable for complex formation. The amount of complex  
35 formation may be quantitated by various methods, preferably by photometric means. Antibodies specific for the mutant ion channels will only bind to individuals

expressing the said mutant ion channels and not to individuals expressing only wild-type channels (ie normal individuals). This establishes the basis for diagnosing the disorder.

5       Once an individual has been diagnosed with a disorder, effective treatments can be initiated as described above. Treatments can be directed to amend the combination of ion channel subunit mutations or may be directed to one mutation.

10

#### Microarray

      In further embodiments, complete cDNAs, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used  
15 as probes in a microarray. The microarray can be used to diagnose epilepsy, as well as other disorders associated with ion channel dysfunction, through the identification of genetic variants, mutations, and polymorphisms in the ion channel subunits that form part of the invention, to  
20 understand the genetic basis of a disorder, or can be used to develop and monitor the activities of therapeutic agents.

      According to a further aspect of the present invention, tissue material obtained from animal models  
25 generated as a result of the identification of specific ion channel subunit human mutations (see below), particularly those disclosed in the present invention, can be used in microarray experiments. These experiments can be conducted to identify the level of expression of  
30 specific ion channel subunits, or any cDNA clones from whole-tissue libraries, in diseased tissue as opposed to normal control tissue. Variations in the expression level of genes, including ion channel subunits, between the two tissues indicates their possible involvement in the  
35 disease process either as a cause or consequence of the original ion channel subunit mutation present in the animal model. These experiments may be used to determine

gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents. Microarrays may be prepared, used, and analyzed using methods known in the art. (For example, see Schena et al., 1996; Heller et al., 1997).

#### Transformed Hosts

The present invention also provides for the production of genetically modified (knock-out, knock-in and transgenic), non-human animal models transformed with nucleic acid molecules containing the novel ion channel mutations or variants as laid out in Table 1. These animals are useful for the study of the function of ion channels, to study the mechanisms by which combinations of mutations in ion channel subunits interact to give rise to disease and the effects of these mutations on tissue development, for the screening of candidate pharmaceutical compounds, for the creation of explanted mammalian cell cultures which express mutant ion channels or combinations of mutant ion channels, and for the evaluation of potential therapeutic interventions.

Animal species which are suitable for use in the animal models of the present invention include, but are not limited to, rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates such as monkeys and chimpanzees. For initial studies, genetically modified mice and rats are highly desirable due to the relative ease in generating knock-in, knock-out or transgenics of these animals, their ease of maintenance and their shorter life spans. For certain studies, transgenic yeast or invertebrates may be suitable and preferred because they allow for rapid screening and provide for much easier handling. For longer term studies, non-human primates may be desired due to their similarity with humans.



To create an animal model for a mutated ion channel, or an animal model incorporating a combination of mutations, several methods can be employed. These include, but are not limited to, generation of a specific mutation in a homologous animal gene, insertion of a wild type human gene and/or a humanized animal gene by homologous recombination, insertion of a mutant (single or multiple) human gene as genomic or minigene cDNA constructs using wild type or mutant or artificial promoter elements, or insertion of artificially modified fragments of the endogenous gene by homologous recombination. The modifications include insertion of mutant stop codons, the deletion of DNA sequences, or the inclusion of recombination elements (lox p sites) recognized by enzymes such as Cre recombinase.

To create transgenic mice in order to study gain of gene function in vivo, any mutant ion channel subunit gene of the invention can be inserted into a mouse germ line using standard techniques such as oocyte microinjection. Gain of gene function can mean the over-expression of a gene and its protein product, or the genetic complementation of a mutation of the gene under investigation. For oocyte injection, one or more copies of the mutant gene can be inserted into the pronucleus of a just-fertilized mouse oocyte. This oocyte is then reimplanted into a pseudo-pregnant foster mother. The live-born mice can then be screened for integrants using analysis of tail DNA for the presence of the relevant human ion channel subunit gene sequence. The transgene can be either a complete genomic sequence injected as a YAC, BAC, PAC or other chromosome DNA fragment, a cDNA with either the natural promoter or a heterologous promoter, or a minigene containing all of the coding region and other elements found to be necessary for optimum expression.

To generate knock-out mice or knock-in mice, gene targeting through homologous recombination in mouse embryonic stem (ES) cells may be applied. Knock-out mice

are generated to study loss of gene function *in vivo* while knock-in mice (which are preferred) allow the study of gain of function or to study the effect of specific gene mutations. Knock-in mice are similar to transgenic mice  
5 however the integration site and copy number are defined in the former.

For knock-out mouse generation, gene targeting vectors can be designed such that they delete (knock-out) the protein coding sequence of the relevant ion channel  
10 subunit gene in the mouse genome. In contrast, knock-in mice can be produced whereby a gene targeting vector containing the relevant ion channel subunit gene can integrate into a defined genetic locus in the mouse genome. For both applications, homologous recombination is  
15 catalysed by specific DNA repair enzymes that recognise homologous DNA sequences and exchange them via double crossover.

Gene targeting vectors are usually introduced into ES cells using electroporation. ES cell integrants are then  
20 isolated via an antibiotic resistance gene present on the targeting vector and are subsequently genotyped to identify those ES cell clones in which the gene under investigation has integrated into the locus of interest. The appropriate ES cells are then transmitted through the  
25 germline to produce a novel mouse strain.

In instances where gene ablation results in early embryonic lethality, conditional gene targeting may be employed. This allows genes to be deleted in a temporally and spatially controlled fashion. As above, appropriate ES  
30 cells are transmitted through the germline to produce a novel mouse strain, however the actual deletion of the gene is performed in the adult mouse in a tissue specific or time controlled manner. Conditional gene targeting is most commonly achieved by use of the cre/lox system. The  
35 enzyme cre is able to recognise the 34 base pair loxP sequence such that loxP flanked (or floxed) DNA is recognised and excised by cre. Tissue specific cre

expression in transgenic mice enables the generation of tissue specific knock-out mice by mating gene targeted floxed mice with cre transgenic mice. Knock-out can be conducted in every tissue (Schwenk et al., 1995) using the  
5 'deleter' mouse or using transgenic mice with an inducible cre gene (such as those with tetracycline inducible cre genes), or knock-out can be tissue specific for example through the use of the CD19-cre mouse (Rickert et al., 1997).

10 Once knock-in animals have been produced which contain a specific mutation in a particular ion channel subunit, mating combinations may be initiated between such animals so as to produce progeny containing combinations of two or more ion channel mutations. These animals  
15 effectively mimic combinations of mutations that are proposed to cause human IGE cases. These animal models can subsequently be used to study the extent and mechanisms of disease as related to the mutated ion channel combinations, as well as for the screening of candidate  
20 therapeutic compounds.

According to still another aspect of the invention there is provided the use of genetically modified non-human animals as described above for the screening of candidate pharmaceutical compounds (see drug screening  
25 above). These animals are also useful for the evaluation (eg therapeutic efficacy, toxicity, metabolism) of candidate pharmaceutical compounds, including those identified from the invention as described above, for the treatment of epilepsy as well as other as other disorders  
30 associated with ion channel dysfunction as described above.

It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of  
35 these documents forms part of the common general knowledge in the art, in Australia or in any other country.

Throughout this specification and the claims, the

words "comprise", "comprises" and "comprising" are used in a non-exclusive sense, except where the context requires otherwise.

5 It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this  
10 specification.

#### Brief Description of the Drawings

Preferred forms of the invention will now be described, by way of example only, with reference to the  
15 following examples and the accompanying drawings, in which:

Figure 1 provides an example of ion channel subunit stoichiometry and the effect of multiple versus single ion channel subunit mutations. Figure 1A: A typical channel  
20 may have five subunits of three different types. Figure 1B: In outbred populations complex diseases such as idiopathic generalized epilepsies may be due to mutations in two (or more) different subunit genes. Because only one allele of each subunit gene is abnormal, half the  
25 expressed subunits will have the mutation. Figure 1C: In inbred populations, both alleles of a single subunit gene will be affected, so all expressed subunits will be mutated. Figure 1D: Autosomal dominant disorders can be attributed to single ion channel subunit mutations that  
30 give rise to severe functional consequences.

Figure 2 represents the location of mutations identified in the KCNQ2 ion channel subunit constituting the potassium channel. M: Missense mutation; T: Truncation mutation; F: Frameshift mutation; S: Splice site mutation.

35 Figure 3 provides examples of epilepsy pedigrees where mutation profiles of ion channel subunits for individuals constituting the pedigree have begun to be

determined. These examples have been used to illustrate how the identification of novel ion channel subunit mutations and variations in IGE individuals can combine to give rise to the disorder.

5        Figure 4 shows the results of yeast two-hybrid analysis of R353G and L619R KCNQ2 mutants. Yeast were transformed with the empty DB (BAIT) plasmid (DBLeu), DB-Q2C wt, DB-Q2C R353G mutant or the DB-Q2 L619R mutant as indicated in A and the AD-CaM (TARGET) vector was  
10 introduced by gap-repair. Yeast control strains (Invitrogen™) were included on all plates for comparison. Control 1 has no interaction. Control 2 has a weak interaction. Control 3 has a moderately strong interaction. Control 4 has a strong interaction and  
15 control 5 has a very strong interaction. B. Growth of transformed yeast and controls on -leu -tryp selection. Yeast can grow on -leu if they contain the DB plasmid, and -tryp if they have AD plasmid. C. Growth of transformed yeast and controls on -leu -tryp -his +40mM 3AT after  
20 48hrs. Yeast can grow on -his+3AT if the his reporter gene is activated by interaction between the BAIT and TARGET plasmids. D-F. LacZ Filter assay for interaction between BAIT and TARGET plasmids, photos taken after 2hrs (D), 7hrs (E) and 24hrs (F). Activation of the  $\beta$ -galactosidase  
25 reporter gene by interaction of the BAIT and TARGET plasmids leads to the dark appearance of colonies.

Figure 5 shows the results of CaM affinity experiments with the R353G and L619R KCNQ2 mutants. The chart below shows the values from the CPRG assay for  $\beta$ -galactosidase activity as a measure of KCNQ2C-CaM binding  
30 efficiency. The area of each bar in the chart equates to the CaM binding efficiency of the BAIT. Broken lines indicate statistical comparison by Student's t test \*  $P < 0.01$ , \*\*  $P < 0.001$ .

35

Modes for Performing the Invention

Potassium channels are the most diverse class of ion channel. The *C. elegans* genome encodes about 80 different potassium channel genes and there are probably more in mammals. About ten potassium channel genes are known to be mutated in human disease and include four members of the KCNQ gene sub-family of potassium channels. KCNQ proteins have six transmembrane domains, a single P-loop that forms the selectivity filter of the pore, a positively charged fourth transmembrane domain that probably acts as a voltage sensor, and intracellular amino and carboxy termini. The C-terminus is long and contains a conserved "A domain" followed by a short stretch thought to be involved in subunit assembly.

Four KCNQ subunits are thought to combine to form a functional potassium channel. All five known KCNQ proteins can form homomeric channels *in vitro* and the formation of heteromers appears to be restricted to certain combinations. For instance KCNQ2 and KCNQ3, which are predominantly expressed in the central nervous system, form a heteromultimeric channel that mediates the neuronal muscarinic-regulated current (M-current), also known as the M-channel (or M-type K<sup>+</sup> channel). The M-current is a slowly activating, non-inactivating potassium conductance known to regulate neuronal excitability by determining the firing properties of neurons and their responsiveness to synaptic input (Wang et al., 1998). Because it is the only current active at voltages near the threshold for action potential initiation, the M-current has a major impact on neuronal excitability.

Sodium (the alpha subunit) and calcium channels are thought to have evolved from the potassium channel subunit, and they each consist of four domains covalently linked as the one molecule, each domain being equivalent to one of the subunits that associate to form the potassium channel. Each of the four domains of the sodium and calcium channels are comprised of six transmembrane

segments.

Voltage-gated sodium channels are required to generate the electrical excitation in neurones, heart and skeletal muscle fibres, which express tissue specific isoforms. Sodium channels are heteromers of a pore forming alpha subunit and a modulatory beta-1 subunit, with an additional beta-2 subunit in neuronal channels. Ten genes encoding sodium channel alpha subunits and 3 genes encoding different beta subunits have so far been identified. The beta subunits of the sodium channels do not associate with the alpha subunits to form any part of the pore, they do however affect the way the alpha pore forming subunit functions.

As with sodium channels, calcium channels consist of a single pore forming alpha subunit, of which at least six types have been identified to date, and several accessory subunits including four beta, one gamma and one alpha2-delta gene. Many of these subunits also encode multiple splice variants adding to the diversity of receptor subunits of this family of ion channels.

The ion channels in the nAChR/GABA super family show a theoretical pentameric channel. Gamma-Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the central nervous system. GABA-ergic inhibition is mediated by two major classes of receptors, type A (GABA-A) and type B (GABA-B). GABA-B receptors are members of the class of receptors coupled to G-proteins and mediate a variety of inhibitory effects via secondary messenger cascades. GABA-A receptors are ligand-gated chloride channels that mediate rapid inhibition.

The GABA-A channel has 16 separate, but related, genes encoding subunits. These are grouped on the basis of sequence identity into alpha, beta, gamma, delta, epsilon, theta and pi subunits. There are six alpha subunits ( $\alpha 1$ - $\alpha 6$ ), three beta subunits ( $\beta 1$ - $\beta 3$ ) and three gamma subunits ( $\gamma 1$ - $\gamma 3$ ). Each GABA-A receptor comprises five subunits which may, at least in theory, be selected from any of these

subunits.

Neuronal nicotinic acetylcholine receptors (nAChRs) consist of heterologous pentamers comprising various combinations of alpha subunits or alpha and beta subunits (α2-α9; β2-β4). The alpha subunits are characterised by adjacent cysteine residues at amino acid positions 192 and 193, and the beta subunits by the lack of these cysteine residues. They are ligand-gated ion channels differentially expressed throughout the brain to form physiologically and pharmacologically distinct receptors hypothesised to mediate fast, excitatory transmission between neurons of the central nervous system or to modulate neurotransmission from their presynaptic position.

In chicken and rat, the predominant nAChR subtype is composed of alpha-4 and beta-2 subunits. The transmembrane 2 (M2) segments of the subunits are arranged as alpha helices and contribute to the walls of the neurotransmitter-gated ion channel. The alpha helices appear to be kinked and orientated in such a way that the side chains of the highly conserved M2-leucine residues project inwards when the channel is closed. ACh is thought to cause a conformational change by altering the association of the amino acid residues of M2. The opening of the channel seems to be due to rotations of the gate forming side chains of the amino acid residues; the conserved polar serines and threonines may form the critical gate in the open channel.

#### Example 1: Identification of mutations in ion channels

Previous studies by reference (Wallace et al., 1998; PCT/AU01/00581; Wallace et al., 2001b; Australian patent AU-B-56247/96; Steinlein et al., 1995; PCT/AU01/00541; Phillips et al., 2001; PCT/AU01/00729; PCT/AU01/01648; PCT/AU02/00910; Wallace et al., 2001a, the disclosures of which are incorporated herein by reference) have identified mutations in a number of ion channel subunits



associated with epilepsy. These include ion channel subunits of voltage-gated (eg SCN1A, SCN1B, KCNQ2, KCNQ3) or ligand-gated (eg CHRNA4, CHRNB2, GABRG2, GABRD) types. To identify further mutations in ion channel genes, subunits which comprise the ion channels were screened for molecular defects in epilepsy patients.

Human genomic sequence available from the Human Genome Project was used to characterize the genomic organisation for each subunit gene. Each gene was subsequently screened for sequence changes using single strand conformation polymorphism (SSCP) analysis in a large sample of epileptics with common sporadic IGE subtypes eg juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE) and epilepsy with generalized tonic-clonic seizures (TCS). Clinical observations can then be compared to the molecular defects characterized in order to establish the combinations of mutant subunits involved in the various disease states, and therefore to provide validated drug targets for each of these disease states. This will provide a basis for novel drug treatments directed at the genetic defects present in each patient.

The coding sequence for each of the ion channel subunits was aligned with human genomic sequence present in available databases at the National Centre for Biotechnology Information (NCBI). The BLASTN algorithm was typically used for sequence alignment and resulted in the genomic organisation (intron-exon structure) of each gene being determined. Where genomic sequence for an ion channel subunit was not available, BACs or PACs containing the relevant ion channel subunit were identified through screening of high density filters containing these clones and were subsequently sequenced.

Availability of entire genomic sequence for each ion channel subunit facilitated the design of intronic primers spanning each exon. These primers were used for both high throughput SSCP screening and direct DNA sequencing.

Example 2: Sample preparation for SSCP screening

A large collection of individuals affected with epilepsy have undergone careful clinical phenotyping and additional data regarding their family history has been collated. Informed consent was obtained from each individual for blood collection and its use in subsequent experimental procedures. Clinical phenotypes incorporated classical IGE cases as well as GEFS+ and febrile seizure cases.

DNA was extracted from collected blood using the QIAamp DNA Blood Maxi kit (Qiagen) according to manufacturers specifications or through procedures adapted from Wyman and White (1980). Stock DNA samples were kept at a concentration of 1 ug/ul.

In preparation for SSCP analysis, samples to be screened were formatted into 96-well plates at a concentration of 30 ng/ul. These master plates were subsequently used to prepare exon specific PCR reactions in the 96-well format.

Example 3: Identification of sequence alterations in ion channel genes

SSCP analysis of specific ion channel exons followed by sequencing of SSCP bandshifts was performed on individuals constituting the 96-well plates to identify sequence alterations.

Primers used for SSCP were labelled at their 5' end with HEX and typical PCR reactions were performed in a total volume of 10 µl. All PCR reactions contained 67 mM Tris-HCl (pH 8.8); 16.5 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 6.5 µM EDTA; 1.5 mM MgCl<sub>2</sub>; 200 µM each dNTP; 10% DMSO; 0.17 mg/ml BSA; 10 mM β-mercaptoethanol; 5 µg/ml each primer and 100 U/ml Taq DNA polymerase. PCR reactions were performed using 10 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds followed by 25 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. A final

extension reaction for 10 minutes at 72°C followed.

Twenty µl of loading dye comprising 50% (v/v) formamide, 12.5 mM EDTA and 0.02% (w/v) bromophenol blue were added to completed reactions which were subsequently run on non-denaturing 4% polyacrylamide gels with a cross-linking ratio of 35:1 (acrylamide:bis-acrylamide) and containing 2% glycerol. Gel thickness was 100µm, width 168mm and length 160mm. Gels were run at 1200 volts and approximately 20mA, at 22°C and analysed on the GelScan 2000 system (Corbett Research, Australia) according to manufacturers specifications.

PCR products showing a conformational change were subsequently sequenced. This first involved re-amplification of the amplicon from the relevant individual (primers used in this instance did not contain 5' HEX labels) followed by purification of the PCR amplified templates for sequencing using QiaQuick PCR preps (Qiagen) based on manufacturers procedures. The primers used to sequence the purified amplicons were identical to those used for the initial amplification step. For each sequencing reaction, 25 ng of primer and 100 ng of purified PCR template were used. The BigDye sequencing kit (ABI) was used for all sequencing reactions according to the manufacturers specifications. The products were run on an ABI 377 Sequencer and analysed using the EditView program.

Table 1 shows the novel sequence changes identified in the ion channel subunits screened.

#### Example 4: Digenic model examples

In some instances a single mutation in an ion channel alone is insufficient to give rise to an epilepsy phenotype. However combinations of mutations each conferring a subtle change of function to an ion channel, as proposed by the digenic model (PCT/AU01/00872), may be sufficient to produce an epilepsy phenotype.

Using the mutations and variations in ion channel

subunits that form part of this invention, the digenic model may be validated through a parametric analysis of large families in which two abnormal alleles co-segregate by chance to identify mutations which act co-operatively to give an epilepsy phenotype. It is envisaged that the strategy of careful clinical phenotyping in these large families, together with a linkage analysis based on the digenic hypothesis will allow identification of the mutations in ion channels associated with IGEs. If molecular genetic studies in IGE are successful using the digenic hypothesis, such an approach might serve as a model for other disorders with complex inheritance.

The digenic hypothesis predicts that the closer the genetic relationship between affected individuals, the more similar the sub-syndromes, consistent with published data (Italian League Against Epilepsy Genetic Collaborative Group, 1993). This is because more distant relatives are less likely to share the same combinations of mutated subunits.

Identical twins have the same pair of mutated subunits and the same minor alleles so the sub-syndromes are identical. Affected sib-pairs, including dizygous twins, with the same sub-syndrome would also have the same pair of mutated subunits, but differences in minor alleles would lead to less similarity than with monozygous twins. Some sib-pairs and dizygous twins, have quite different sub-syndromes; this would be due to different combinations of mutated subunits, when the parents have more than two mutated alleles between them.

A special situation exists in inbred communities that parallels observations on autosomal recessive mouse models. Here the two mutated alleles of the digenic model are the same and thus result in a true autosomal recessive disorder. Because all affected individuals have the same pair of mutated alleles, and a similar genetic background, the phenotypes are very similar.

In outbred communities approximately 1% of the

population would have IGE genotypes (2 mutated alleles) and 0.3% would clinically express IGE. Most of these would have mutations in two different channel subunits. In such communities most cases would appear "sporadic" as the risk to first degree relatives would be less than 10%.

For example, let there be three IGE loci (A,B,C) and let the frequency of abnormal alleles ( $a^*, b^*, c^*$ ) at each locus be .027 and of normal alleles ( $a, b, c$ ) be .973. Then, the distribution of genotypes  $aa^*, a^*a, a^*a^*$  and  $aa$  at locus A will be .0263 ( $.027 \times .973$ ), .0263, .0007 and .9467 respectively, and similarly for loci B and C. In this population .8485 will have no mutated alleles ( $.9467^3$ ), .1413 will have one mutated allele ( $a^*$  or  $b^*$  or  $c^*$ ;  $.0263 \times .9467^2 \times 6$ ), .0098 will have two abnormal alleles (.0020 two same abnormal alleles, .0078, two different abnormal alleles) and 0.00037 will have more than two abnormal alleles. Thus in this population .01, or 1%, will have two or more abnormal alleles (IGE genotype), and the total abnormal allele frequency will be .08 ( $3 \times .027$ ).

To determine the familial risks and allele patterns in affected pairs, the frequency distribution of population matings and the percentage of children with 2 or more abnormal alleles must be determined. The frequency of matings with no abnormal alleles ( $0 \times 0$ ) is .72 ( $.8485^2$ ), for  $1 \times 0$  and  $0 \times 1$  matings .24 ( $2 \times .8485 \times .1413$ ), for a  $1 \times 1$  mating .020, and for  $2 \times 0$  and  $0 \times 2$  matings .0166 etc. From this distribution of matings the frequency of children with 2 or more abnormal alleles can be shown to be .01. For example, the  $0 \times 2$  and  $2 \times 0$  matings contribute .0033 of this .01 frequency ( $.0166$  [mating frequency]  $\times .2$  [chance of that mating producing a child with 2 or more abnormal alleles]).

To determine parental risk it can be shown that of children with 2 abnormal alleles (IGE genotype), .49 derive from  $1 \times 1$  matings where no parent is affected, .33 derive from a  $2 \times 0$  and  $0 \times 2$  matings etc. For the  $2 \times 0$

and 0 x 2 matings, half the parents have IGE genotypes and contribute .16 (.33/2) to the parental risk with the total parental risk of an IGE genotype being .258. The other matings that contribute to affected parent-child pairs are  
5 2 x 1, 1 x 2, 3 x 0, 0 x 3 etc.

The sibling risk of an IGE genotype is .305. For example 2 x 0 and 0 x 2 matings contributed .08 to the sibling risk (.33[fraction of children with 2 abnormal alleles] x .25[the chance of that mating producing a child  
10 with 2 or more abnormal alleles]). Similarly the offspring risk was determined to be .248 by mating individuals with 2 abnormal alleles with the general population. Thus at 30% penetrance the risk for IGE phenotype for parents of a proband is .077, for siblings .091, and for offspring  
15 .074.

It can be shown that affected sib pairs share the same abnormal allele pair in 85% of cases. This is because of all affected sib pairs 44% derive from 1 x 1 matings and 23% from 0 x 2 and 2 x 0 matings where all affected  
20 siblings have the same genotype. In contrast, 24% derive from 1 x 2 matings and 9% from 3 x 1 and 2 x 2 matings etc where affected sibling genotypes sometimes differ.

For affected parent-child pairs, genotypes are identical in only 58%. Of affected parent child pairs, 43%  
25 derive from 0 x 2 matings where genotypes are identical, whereas 38% derive from 0 x 3 and 17% from 1 x 2 where the majority of crosses yield different affected genotypes.

Based on the digenic model it has been postulated that most classical IGE and GEFS' cases are due to the  
30 combination of two mutations in multi-subunit ion channels. These are typically point mutations resulting in a subtle change of function. The critical postulate is that two mutations, usually, but not exclusively, in different subunit alleles ("digenic model"), are required  
35 for clinical expression of IGE.

The hypothesis that similar phenotypes can be caused by the combination of mutations in two (or more) different

subunits (outbred communities), or by the same mutation in two (or more) alleles of the same subunit (inbred communities), may seem implausible. However, applying the digenic hypothesis to the theoretical pentameric channel shown in Figure 1, in outbred communities IGE will be due to subunit combinations such as  $\alpha^*\alpha\beta^*\beta\Delta$ ,  $\alpha^*\alpha\beta\beta\Delta^*$  or  $\alpha\alpha\beta^*\beta\Delta^*$  (mutated subunits indicated by \*). In inbred communities  $\alpha^*\alpha^*\beta\beta\Delta$  or  $\alpha\alpha\beta^*\beta^*\Delta$  combinations might cause IGE phenotypes. We assume that the mutations will not cause reduced expression of the alleles and that the altered ion channel excitability, and consequent IGE phenotype, caused by mutations in two different alleles is similar to that caused by the same mutation in both alleles of one subunit. Finally, subunit mutations with more severe functional consequences (eg breaking a disulphide bridge in SCN1B or amino acid substitution in the pore forming regions of SCN1A for GEFS<sup>+</sup>) cause autosomal dominant generalized epilepsies with a penetrance of 60-90%. Such "severe" mutations are rare (allele frequency <0.01%) and are infrequent causes of GEFS<sup>+</sup>. They very rarely, or perhaps never, cause classical IGE.

The relative separate segregation of classical IGE and GEFS<sup>+</sup> phenotypes is an anecdotal clinical observation of ours (Singh et al., 1999), although the separation is not absolute. The separation is supported by previous family and EEG studies of Dose and colleagues who described "type A" and "type B" liabilities which we may approximate the GEFS<sup>+</sup> and classical IGE groupings respectively (Dose and Baier, 1987).

The digenic model predicts that affected sib pairs will share the same genes in 85% of cases whereas they will have at least one different allele in the remaining 15%. In contrast, only 58% of parent-child pairs share the same alleles in a 3 locus model. Thus there should be greater similarity of syndromes between sibling pairs than parent-child pairs. This would be most objectively measured by age of onset and seizure types.

Estimates for the risk of febrile seizures or IGE in relatives vary. The estimates range from 5%-10% for siblings, 4%-6% for offspring, 3%-6% for parents, and 2-3% for grandparents. Underestimation may occur because IGE  
5 manifest in youth, and parents and particularly grandparents may be unaware of seizures in themselves in younger years. This is particularly true where there was stigma associated with epilepsy and where the epilepsy may have been mild and unrecognized. Underestimation of  
10 sibling and offspring risks occurs when unaffected young children are counted, some of whom will develop IGE in adolescence. Overestimation may occur with misdiagnosis of seizures or inclusion of seizures unrelated to IGE (e.g. due to trauma or tumors)

15 In autosomal dominant models the risk to affected relatives reduces proportionally (50% for first degree relatives, 25% for second degree etc). For all oligogenic or polygenic models the risk decreases more quickly. For a digenic model with three loci, the risks are 9.1% for  
20 siblings, 7.4% for offspring, 7.7% for parents. Rigorous measurement of the familial recurrence rates, with careful phenotyping and age-corrected risk estimates could be compared with the predictions from the digenic model, and it is proposed to do this.

25 There is a small amount of information on IGE families regarding haplotype distribution. For example, there is some evidence for a locus on 8q as determined by parametric linkage in a single family (Fong et al., 1998) and by non-parametric analysis in multiple small families  
30 (Zara et al., 1995). Interestingly, in the latter study the 8q haplotype not infrequently came from the unaffected parent. This would be quite compatible with the digenic model and evaluation of other data sets in this manner could be used to test the hypothesis, and it is proposed  
35 to do this.

Following the analysis of one large family with epilepsy where the two main phenotypes were childhood



absence epilepsy (CAE) and febrile seizures (FS), the inheritance of FS was found to be autosomal dominant and the penetrance 75%. However the inheritance of CAE in this family was not simple Mendelian, but suggestive of complex inheritance with the involvement of more than one gene. The power of this large family was used to explore the complex genetics of CAE further.

Linkage analysis on this family in which individuals with CAE, FS and FS+ were deemed affected led to the detection of linkage on chromosome 5q and identification of a mutation in the GABRG2 gene (R43Q) which is localised to this region (Wallace et al., 2001a; PCT/AU01/00729). All 10 tested individuals with FS alone in this family had this mutation and 7 CAE affected individuals in this family also had the mutation. To test the digenic model of IGEs in the CAE affected individuals, the whole genome screen of this family was reanalysed with only individuals with CAE considered affected. Linkage analysis was performed using FASTLINK v4.0, two-point lod scores were calculated assuming 50% penetrance and a 2% phenocopy rate and individuals with FS or FS+ were coded as unknown. Markers producing a lod score greater than 1 were reanalysed without a phenocopy rate and at the observed penetrance for CAE in this family (30%). Results from the analysis revealed significant linkage to chromosome 14q22-q23 (lod 3.4). This provides strong evidence for a second locus segregating with CAE affected individuals in this family. While the GABRG2 mutation is sufficient to cause FS, the CAE phenotype is thought to be due to both the GABRG2 mutation and a mutation occurring in a gene mapping to the 14q locus, as proposed by the digenic model.

For the application of the digenic model to sporadic cases of IGE and affected individuals belonging to smaller families in which genotyping and linkage analysis is not a feasible approach to disease gene identification, direct mutation analysis of ion channel genes in these individuals has been carried out as described above. In

Table 1 there is provided an indication of novel genetic alterations so far identified through mutation analysis screening of these individuals. Figure 2 provides an example to indicate where some of these mutations have  
5 occurred with respect to the potassium channel KCNQ2 gene.

The identification of novel mutations and variations in ion channel subunits in IGE individuals provides resources to further test the digenic hypothesis and mutation profiles are starting to accumulate for a number  
10 of subunit changes that are observed in the same individuals. Figure 3 provides results from some of these profiles.

Figure 3A shows a 3 generation family in which individual III-1 has myoclonic astatic epilepsy and contains a N43del mutation in the SCN3A gene as well as an A1067T mutation in the SCN1A gene. Individual I-1 also has the SCN3A mutation but alone this mutation is not sufficient to cause epilepsy in this individual. The SCN3A mutation has likely been inherited from the grandfather  
20 through the mother, while the SCN1A mutation is likely to arise from the father. Both parents are unaffected but have yet to be screened for the presence of the mutations in these subunits. Individual II-1 is likely to contain an as yet unidentified ion channel subunit mutation acting in co-operation with the SCN3A mutation already identified in  
25 this individual.

Figure 3B is another 3 generation family in which individual III-1 has myoclonic astatic epilepsy due to a combination of the same SCN3A and SCN1A mutations as  
30 above. However, in this family both parents have febrile seizures most likely due to the presence of just one of the mutations in each parent, as proposed by the model. This is in contrast to individuals II-2 and II-3 in Figure 4A who also contain one of the mutations in these genes  
35 each. These individuals are phenotypically normal most likely due to incomplete penetrance of these mutations in each case.

Figure 3C shows a larger multi-generation family in which individual IV-5 has a mutation in both the SCN3A and GABRG2 subunits. In combination, these give rise to severe myoclonic epilepsy of infancy but alone either cause  
5 febrile seizures (GABRG2 mutation in III-3 and IV-4) or are without an effect (SCN3A mutation in III-2) as proposed by the model.

These examples therefore illustrate the digenic model as determined from mutation analysis studies of ion  
10 channel subunits in affected individuals and highlight the need to identify genetic alterations in the genes encoding ion channel subunits.

Example 5: Analysis of ion channels and ion channel  
15 subunits

The structure and function of the mutant ion channels and mutant ion channel subunits of the present invention can be determined using a variety of molecular biological studies. These studies may provide clues as to the  
20 mechanisms by which mutations in ion channel subunits effect the functioning of the ion channel. For instance the identification of proteins that interact with mutant ion channels (or whose interaction is impeded by a mutation in an ion channel subunit) may help determine the  
25 molecular mechanisms that are disrupted as a result of a mutation. Procedures such as the yeast two-hybrid system can be used to discover and identify such interacting proteins.

The principle behind the yeast two-hybrid procedure  
30 is that many eukaryotic transcriptional activators, including those in yeast, consist of two discrete modular domains. The first is a DNA-binding domain that binds to a specific promoter sequence and the second is an activation domain that directs the RNA polymerase II complex to  
35 transcribe the gene downstream of the DNA binding site. Both domains are required for transcriptional activation as neither domain can activate transcription on its own.

In the yeast two-hybrid procedure, the gene of interest or parts thereof (BAIT), is cloned in such a way that it is expressed as a fusion to a peptide that has a DNA binding domain. A second gene, or number of genes, such as those  
5 from a cDNA library (TARGET), is cloned so that it is expressed as a fusion to an activation domain. Interaction of the protein of interest with its binding partner brings the DNA-binding peptide together with the activation domain and initiates transcription of the reporter genes.  
10 The first reporter gene will select for yeast cells that contain interacting proteins (this reporter is usually a nutritional gene required for growth on selective media). The second reporter is used for confirmation and while being expressed in response to interacting proteins it is  
15 usually not required for growth.

#### KCNQ2 interactors

Despite the identification of a number of KCNQ2 mutations responsible for epilepsy, including those of the  
20 present study, the underlying biological mechanisms responsible for the epilepsy remains largely uncharacterized. Towards identifying these mechanisms, the large intracellular C-terminal region of KCNQ2 was screened for interactions with other proteins using the  
25 yeast-two hybrid procedure. The C-terminus accounts for 63% of the KCNQ2 protein and, in common with other KCNQ subunits, contains a conserved 'A domain' (Jentsch, 2000; Schwake et al., 2000) thought to be involved in subunit interactions as well as another distal short conserved  
30 region that has been associated with subunit assembly, at least in KCNQ1 (Jentsch, 2000; Schmitt et al., 2000).

#### A) Yeast-two hybrid analysis

A yeast two-hybrid screen was carried out using the  
35 ProQuest™ Two-Hybrid System with Gateway™ Technology (Invitrogen™) according to manufacturer's directions. A KCNQ2 C-terminal entry (BAIT) clone was generated using

the pENTR Directional TOPO<sup>®</sup> Cloning Kit (Invitrogen<sup>™</sup>). The following primers were designed to amplify the intracellular C-terminal region of KCNQ2 based on the sequence of human KCNQ2 (Genbank accession number NM\_172107): KCNQ2F: 5'-CACCAAGGTTTCAGGAGCAGCACAGG-3' and KCNQ2R: 5'-TCACTTCCTGGGCCCCGGCCAGCC-3'. The 1611 base pair cloned fragment included exon 10a (found in all our amplified clones), corresponding to amino acid 373-382 of the KCNQ2 protein. The extra 30 base pairs (10 amino acids) were included in our numbering. The PCR-product was cloned into the pENTR/D-TOPO<sup>®</sup> vector (Invitrogen<sup>™</sup>) via the TOPO<sup>®</sup> Cloning reaction according to the manufacturer's instructions. Following sequence verification, the KCNQ2 cDNA fragment was then subcloned into pDEST<sup>™</sup>32, the DNA Binding domain (DB) Gateway<sup>™</sup> Destination Vector (Invitrogen<sup>™</sup>).

The ProQuest<sup>™</sup> Two-Hybrid human brain cDNA Library (TARGET) with Gateway<sup>™</sup> technology (ResGen<sup>™</sup>, Invitrogen<sup>™</sup> Corporation) was amplified according to the manufacturer's instructions. Plasmid DNA was purified from the cell pellet using the HiSpeed Plasmid Maxi Kit (Qiagen) according to the manufacturer's instructions.

Both the DBLeu (empty bait vector) and DB-KCNQ2 wild-type (wt) C-term BAITs were transformed into the yeast strain Mav203 and plated onto minimal selective media lacking leucine. A duplicate was carried out where the empty library TARGET (pAD) vector was co-transformed in addition to each BAIT and plated onto minimal selective media lacking leucine (-leu) and tryptophan (-tryp). Yeast control strains (Invitrogen<sup>™</sup>) were included on all plates. Control 1, used as a negative control, contained empty plasmids pPC97 and pPC86. Control 2 had pPC97-RB and pPC86-E2F1, which express a relatively weak interaction. Control 3 contained plasmids encoding the *Drosophila* DP (pPC97) and E2F (pPC86) domains that have a moderately strong interaction, and provide a control for plasmid shuffling. Control 4 contained pPC97-Fos and pPC86-Jun

which express a relatively strong interaction, and control 5 had a pCL1 plasmid encoding full-length GAL4p and empty pPC86 and was used as a positive control.

5 The constructs were tested for self-activation of the *his* and  $\beta$ -gal reporter genes according to Invitrogen™ instructions.

For the yeast-two hybrid screen, competent yeast cells were prepared for each BAIT (DB-KCNQ2 wt C-term construct) to be screened, transformed with 31μg of ProQuest™ Two-Hybrid human brain AD (activation domain)-cDNA Library and plated onto minimal selective media lacking leucine (-leu), tryptophan (-tryp) and histidine (-his) and containing 3-aminotriazole (+3AT). Positive colonies from each screen were PCR-amplified and re-introduced into fresh yeast cells containing the BAIT to re-test for two-hybrid interaction phenotypes. Those giving rise to more than one PCR product or that failed to re-test positively were systematically eliminated. Positives that re-tested were sequenced using the ABI PRISM® BigDye™ Terminators v3.0 technology. Once identified, the sequence of the potential interactor was checked to verify it was in the same translational frame as the Gal4p-AD encoding sequence of the prey construct.

25 Approximately  $3 \times 10^6$  clones from the ProQuest™ Two-Hybrid human brain cDNA Library were screened for interaction with the DB-Q2C wt bait. Among 1039 positive AD-cDNAs recovered, re-tested and subsequently sequenced all were identified as the CALM2 gene, encoding the ubiquitous,  $\text{Ca}^{2+}$ -binding protein, Calmodulin (CaM).

30 The interaction between the C-terminal region of KCNQ2 and CaM has also been reported by other studies (Wen and Levitan, 2002; Yus-Najera et al., 2002; Gamper and Shapiro, 2003). In mammals, the CaM protein is coded by a multigene family consisting of three bona fide members, 35 CALM1, CALM2 and CALM3. Within the non-coding regions of the CaM transcripts, no striking homology is observed, and codon usage is maximally divergent amongst the three CaM

mRNAs that encode an identical protein. It has been hypothesised that the existence of a multigene family provides a tight and complex level of regulatory control at the level of gene expression (Palfi et al., 2002). CaM genes are differentially expressed in the CNS during development and differential regulation of the CaM genes appears necessary to maintain the temporal and spatial fidelity of the CaM protein levels in all subcellular domains. Besides the fundamental housekeeping functions associated with CaM, it is also involved in specialized neuronal functions, such as the synthesis and release of neurotransmitters, neurite extension, long-term potentiation and axonal transport (Palfi et al., 2002).

B) Effect of epilepsy-associated KCNQ2 mutations on the CaM-KCNQ2 interaction

To assess the effect that the C-terminus mutations of the present invention had on CaM binding, two of the identified mutations (R353G and L619R) were introduced into the DB-Q2C construct by mutagenesis and were re-analysed for an interaction with CaM using the yeast two-hybrid procedure.

The following primers were used to incorporate the c1057C→G (R353G) and c1856T→G (L619R) changes into the pDEST<sup>TM</sup>32- KCNQ2 C-terminal bait construct.

R353G F 5'-CGCCACCAACCTCTCGGGCACAGACCTGCACTC-3'  
R353G R 5'-GAGTGCAGGTCTGTGCCCCGAGAGGTTGGTGGCG-3'  
L619R F 5'-CTTGTCCATGGAGAAGAAGCGGGACTTCCTGGTGAATATC-3'  
L619R R 5'-GATATTCACCAGGAAGTCCCGCTTCTTCTCCATGGACAAG-3'

Overlapping PCR products were generated using the TOPO<sup>®</sup> cloning compatible KCNQ2F primer from the initial cloning and the mutagenesis reverse primers, and the KCNQ2R primer from the initial cloning with the mutagenesis forward primers. Products were gel extracted and purified before a second round of PCR using the

initial KCNQ2 F&R primers. These products were also gel extracted before cloning into the pDEST<sup>TM</sup>32 bait vector via the TOPO<sup>®</sup> system (as described above). Mutant baits were sequence verified.

5       The interaction between each DB-Q2C mutant and CaM was then tested by the yeast two-hybrid assay and compared to the interaction with DB-Q2 wt. Three different PCR-amplified CaM positive clones from the initial screen were re-introduced by gap-repair<sup>20</sup> into the prey vector (pPC86)  
10       in the yeast strain expressing either DB-Q2C wt, DB-Q2C mutants or the empty DBLep vector, used as negative control.

      CaM interaction with the DB-Q2C wt and mutants was then assessed by expression of the *HIS3* and *LacZ* reporter  
15       genes.

      The Q2C R353G mutant did not interact with CaM, as seen by no growth on *HIS3* selective plate (Figure 4C) and no blue readout in the *LacZ* filter assay (Figure 4D-F). On the other hand, the DB-Q2C L619R mutant was shown to still  
20       interact with CaM, as seen by growth on *HIS3* selective plate (Figure 4C) and the blue readout in the *LacZ* filter assay. Interestingly, the DB-Q2C L619R mutant showed an even greater growth level on *HIS3* selective plate than the DB-Q2C wt and also appeared to stain faster and more  
25       intensely blue in the *LacZ* filter assay, suggesting a stronger interaction between CaM and this mutant.

      In order to better quantify  $\beta$ -gal activity, a second assay was carried out using the high sensitivity substrate Chlorophenol Red- $\beta$ -D-Galactopyranoside (CPRG) in liquid  
30       culture. The affinity of the DB-Q2C/AD-CaM interaction was measured in terms of units of  $\beta$ -gal activity, with a zero value indicating no expression of the *LacZ* reporter gene, and hence no interaction.

      In the CPRG assay, a value of 0.05 units  $\beta$ -gal  
35       activity (Figure 5) was significantly different from the empty bait vector replicate ( $P < 0.01$ , Student's *t* test), confirming the interaction of the DB-Q2C wt with CaM.



As observed in the LacZ filter assay, the CPRG assay showed a significant difference in the interaction between the Q2C R353G mutant and CaM as compared to the wt replicate ( $P < 0.01$ , Student's *t* test, Figure 4).

5        These results suggest that the R353G mutation alters the structural conformation of the KCNQ2 C-terminal domain such that it is no longer able to bind to CaM and that this single point mutation is sufficient to abolish the interaction. By abolishing CaM binding, the R353G mutation  
10        could lead to an impairment of M-current *in vivo* due to decreased opening of the channel.

      In contrast, the CPRG assay for the L619R Q2C mutant showed a significantly higher level of  $\beta$ -gal activity units (0.26 units) than the wt replicate ( $P < 0.001$ , Student's *t*  
15        test, Figure 5). This finding indicates that the L619R mutation alters the conformation of the protein in a manner that increases CaM binding affinity for the KCNQ2 C-terminal domain by approximately 5-fold. The increased affinity for CaM may affect the ability of the complex to  
20        change conformation normally in response to calcium signalling. Alternatively, the marked increase in binding of CaM to the KCNQ2 L619R mutant channel may be detrimental to the M-channel function via disruption of the normal neuronal inhibitory/excitatory balance,  
25        therefore causing the seizures associated with epilepsy, particularly BFNS. CaM is known to be involved in both the excitatory and inhibitory neurotransmission pathways (Ohya and Botstein, 1994) and it has been proposed that the temporal and spatial restrictions on CaM itself could  
30        enable the tight control of these opposing reactions (Toutenhoofd and Strehler, 2000). Hence, the KCNQ2 L619R mutation could lead to a disruption of the local CaM pool consequently disturbing the finely balanced excitatory and inhibitory neurotransmission systems.

35        These results implicate CaM in the pathogenesis of epilepsy and specifically in the BFNS syndrome. Whilst further work will be required to fully elucidate the

involvement of the KCNQ2-CaM interaction in neuronal excitability and its correlation with idiopathic epilepsy, these data suggest that dysfunction of this interaction leads to aberrant neuronal excitability in some BFNS patients.

The calmodulin gene (and other ion channel interacting genes) may therefore be a target for mutation in epilepsy as well as other disorders associated with ion channel dysfunction. A mutation in an ion channel interacting gene when expressed alone, or when expressed in combination with one or more other ion channel mutations or ion channel interacting gene mutations (based on the digenic model), may give rise to the disorder. The nature of the ion channel interacting genes and proteins can be studied such that these partners can also be targets for drug discovery.

Industrial Applicability

The mutant ion channel receptor subunits of the invention are useful in the diagnosis and treatment of diseases such as epilepsy and disorders associated with ion channel dysfunction including, but not limited to, hyper- or hypo-kalemic periodic paralysis, myotonias, malignant hyperthermia, myasthenia, cardiac arrhythmias, episodic ataxia, migraine, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, anxiety, depression, phobic obsessive symptoms, neuropathic pain, inflammatory pain, chronic/acute pain, Bartter's syndrome, polycystic kidney disease, Dent's disease, hyperinsulinemic hypoglycemia of infancy, cystic fibrosis, congenital stationary night blindness and total colour-blindness.

TABLE 1

Examples of mutations and variations identified in ion channel subunit genes

Subunit Gene	Exon/Intron	DNA Mutation	Amino Acid Change	SEQ ID NOS
<b>Sodium Channel Subunits</b>				
Coding exonic variants - amino acid change				
SCN1B <sup>r</sup>	Exon 3	c254G→A	R85H	1, 63
SCN2A <sup>r</sup>	Exon 6A	c668G→A	R223Q	2, 64
SCN2A <sup>r</sup>	Exon 16	c2674G→A	V892I	3, 65
SCN2A <sup>r</sup>	Exon 17	c3007C→A	L1003I	4, 66
SCN2A <sup>r</sup>	Exon 19	c3598A→G	T1200A	5, 67
SCN2A <sup>r</sup>	Exon 20	c3956G→A	R1319Q	6, 68
Coding exonic variants - no amino acid change				
SCN2A <sup>c</sup>	Exon 12	c1785T→C	-	7
SCN2A <sup>c</sup>	Exon 27	c4919T→A	-	8
Non-coding variants				
SCN1A <sup>c</sup>	Intron 23	IVS23+33G→A	-	9
SCN2A <sup>r</sup>	Intron 7	IVS7+61T→A	-	10
SCN2A <sup>r</sup>	Intron 19	IVS19-55A→G	-	11
SCN2A <sup>r</sup>	Intron 22	IVS22-31A→G	-	12
SCN2A <sup>c</sup>	Intron 2	IVS2-28G→A	-	13
SCN2A <sup>c</sup>	Intron 8	IVS8-3T→C	-	14
SCN2A <sup>c</sup>	Intron 11	IVS11+49A→G	-	15
SCN2A <sup>c</sup>	Intron 11	IVS11-16C→T	-	16
SCN2A <sup>c</sup>	Intron 17	IVS17-71C→T	-	17
SCN2A <sup>c</sup>	Intron 17	IVS17-74delG	-	18
SCN2A <sup>c</sup>	Intron 17	IVS17-74insG	-	19
<b>Nicotinic Acetylcholine Receptor Subunits</b>				
Coding exonic variants - amino acid change				
CHRNA5 <sup>r</sup>	Exon 4	c400G→A	V134I	20, 69
CHRNA2 <sup>c</sup>	Exon 4	c373G→A	A125T	21, 70
CHRNA3 <sup>c</sup>	Exon 2	c110G→A	R37H	22, 71
Coding variants - no amino acid change				
CHRNA2 <sup>c</sup>	Exon 4	c351C→T	-	23
CHRNA2 <sup>c</sup>	Exon 5	c771C→T	-	24
CHRNA3 <sup>c</sup>	Exon 2	c159A→G	-	25
CHRNA3 <sup>c</sup>	Exon 4	c291G→A	-	26
CHRNA3 <sup>c</sup>	Exon 4	c345G→A	-	27
Non-coding variants				
CHRNA2 <sup>c</sup>	Intron 3	IVS3-16C→T	-	28
CHRNA3 <sup>c</sup>	Intron 3	IVS3-5T→C	-	29
CHRNA3 <sup>c</sup>	Intron 4	IVS4+8G→C	-	30

**Potassium Channel Subunits**

**Coding exonic variants - amino acid change**

KCNQ2 <sup>r</sup>	Exon 1	c204-c205insC	K69fsX119	31, 72
KCNQ2 <sup>r</sup>	Exon 1	c1A→G	M1V	32
KCNQ2 <sup>r</sup>	Exon 1	c2T→C	M1T	33
KCNQ2 <sup>r</sup>	Exon 8	c1057C→G	R353G	34, 73
KCNQ2 <sup>r</sup>	Exon 11	c1288C→T	R430X	35, 74
KCNQ2 <sup>r</sup>	Exon 14	c1710A→T	R570S	36, 75
KCNQ2 <sup>r</sup>	Exon 15	c1856T→G	L619R	37, 76

**Non-coding variants**

KCNQ2 <sup>r</sup>	Intron 9	IVS9+(46-48)delCCT	-	38
KCNQ3 <sup>r</sup>	Intron 11	IVS11+43G→A	-	39
KCNQ3 <sup>c</sup>	Intron 12	IVS12+29G→A	-	40

**GABA Receptor Subunits**

**Coding exonic variants - no amino acid change**

GABRB1 <sup>r</sup>	Exon 5	c508C→T	-	41
GABRB1 <sup>r</sup>	Exon 9	c1329G→A	-	42
GABRB1 <sup>c</sup>	Exon 8	c975C→T	-	43
GABRG3 <sup>c</sup>	Exon 8	c995T→C	-	44

**Non-coding variants**

GABRA1 <sup>c</sup>	5' UTR	c-142A→G	-	45
GABRA1 <sup>c</sup>	5' UTR	c-31C→T	-	46
GABRA2 <sup>c</sup>	3' UTR	c1615G→A	-	47
GABRA5 <sup>c</sup>	5' UTR	c-271G→C	-	48
GABRA5 <sup>c</sup>	5' UTR	c-228A→G	-	49
GABRA5 <sup>c</sup>	5' UTR	c-149G→C	-	50
GABRB2 <sup>b</sup>	5' UTR	c-159C→T	-	51
GABRB2 <sup>c</sup>	3' UTR	c1749C→T	-	52
GABRPi <sup>c</sup>	5' UTR	c-101C→T	-	53
GABRB1 <sup>c</sup>	Intron 1	IVS1+24T→G	-	54
GABRB1 <sup>c</sup>	Intron 5	IVS6+72T→G	-	55
GABRB1 <sup>c</sup>	Intron 7	IVS7-34A→G	-	56
GABRB3 <sup>r</sup>	Intron 1	IVS1-14C→T	-	57
GABRB3 <sup>r</sup>	Intron 7	IVS7+58delAA	-	58
GABRD <sup>r</sup>	Intron 6	IVS6+132insC	-	59
GABRD <sup>r</sup>	Intron 6	IVS6+130insC	-	60
GABRD <sup>r</sup>	Intron 6	IVS6+73del	-	61
CGCGCCACCGCCCTTCCGCG				
GABRG3 <sup>c</sup>	Intron 8	IVS8-102C→T	-	62

Note: <sup>r</sup> Mutations or variations only occurring in individuals with epilepsy; <sup>b</sup> Variant seen only in normal control samples; <sup>c</sup> Mutations or variants seen in individuals with epilepsy as well as normal control samples. The KCNQ2 numbering is based on the large isoform (inclusion of exon 10a). The numbering of exons and introns for SCN2A is based on the publication of Kasai et al., 2001.

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- 25 Dated this 7th day of August 2003  
BIONOMICS LIMITED  
By their Patent Attorneys  
GRIFFITH HACK  
Fellows Institute of Patent and  
30 Trade Mark Attorneys of Australia

Abstract

A method of identifying a subject predisposed to a disorder associated with ion channel dysfunction, comprising ascertaining whether at least one of the genes  
5 encoding ion channel subunits in said subject has undergone a mutation event as set forth in one of SEQ ID Numbers: 1-62.

Figure 1

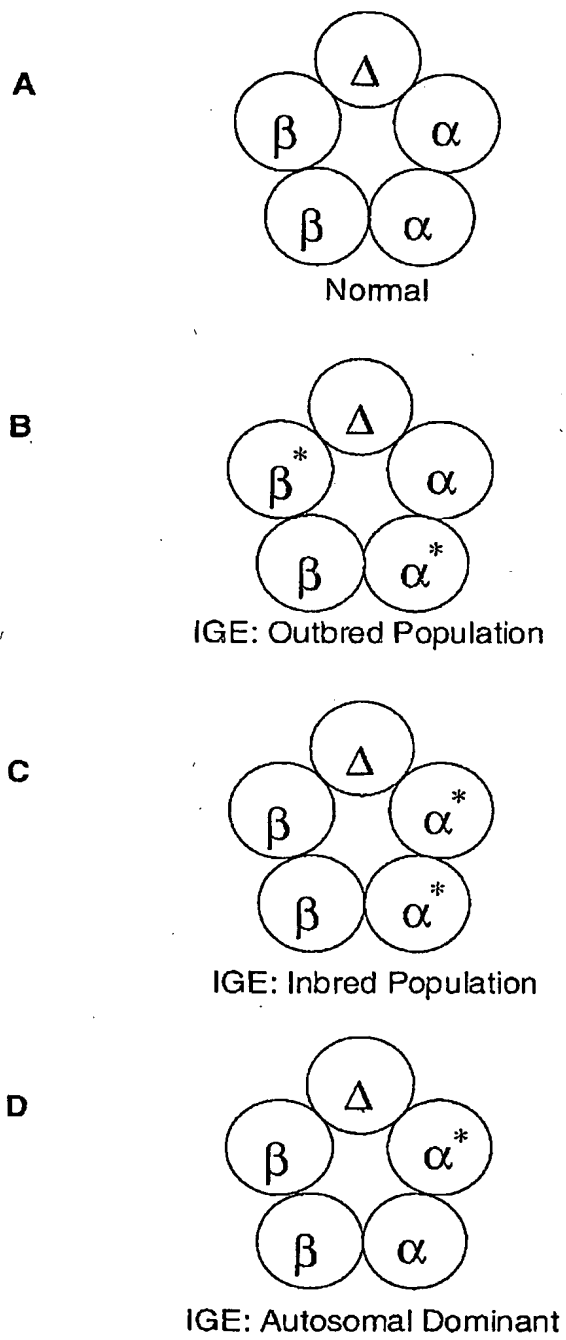


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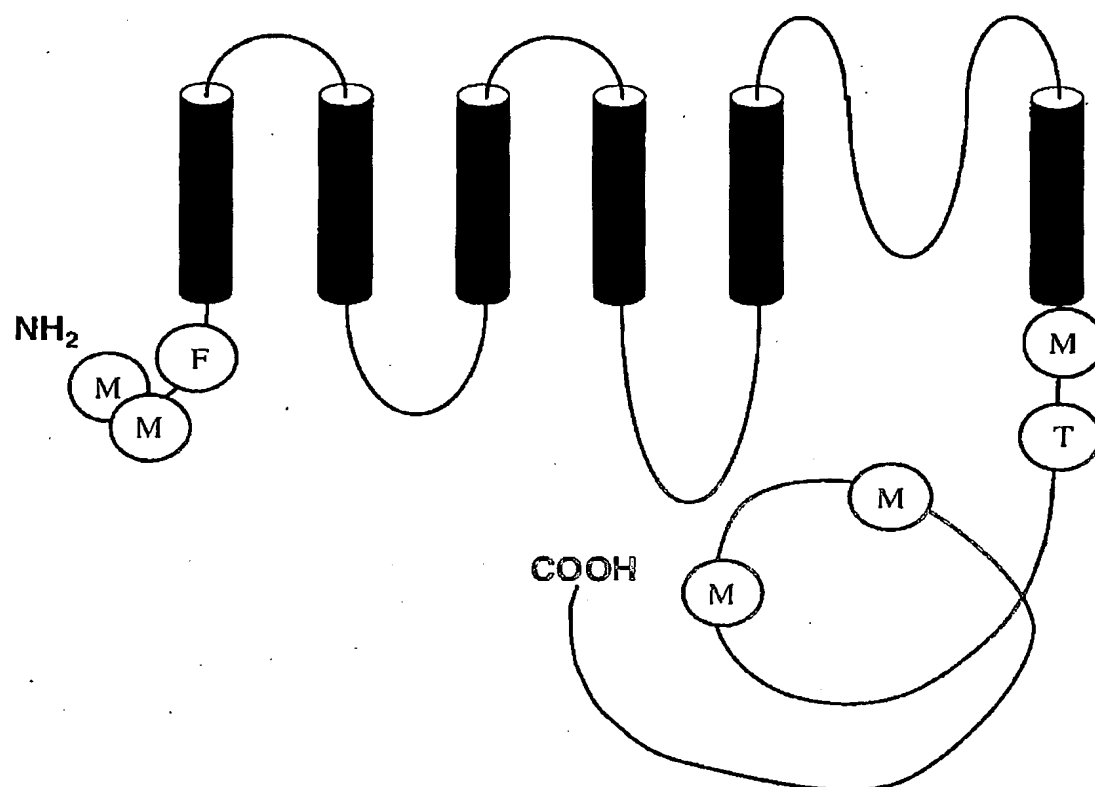
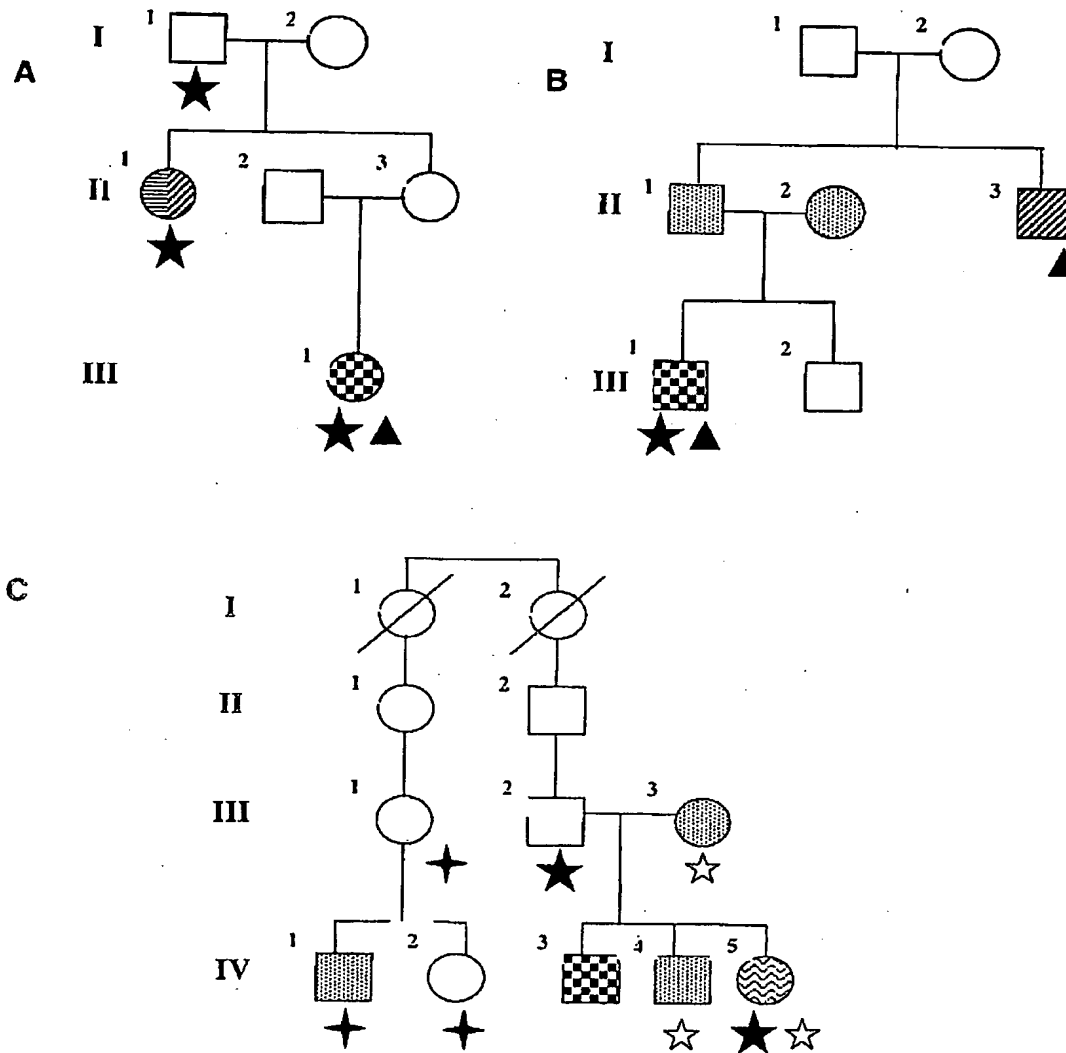


Figure 3












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|  | Myoclonic Astatic Epilepsy           |  | G1050S SCN8A |
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Figure 4

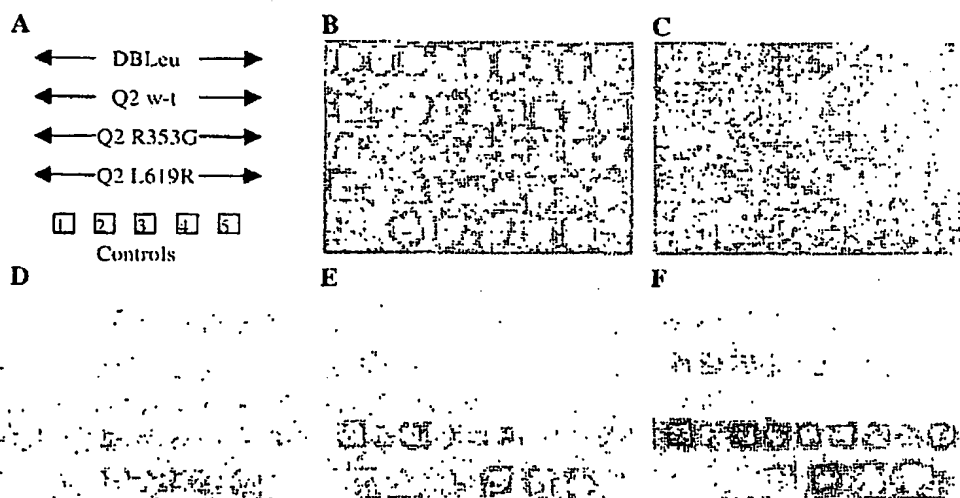
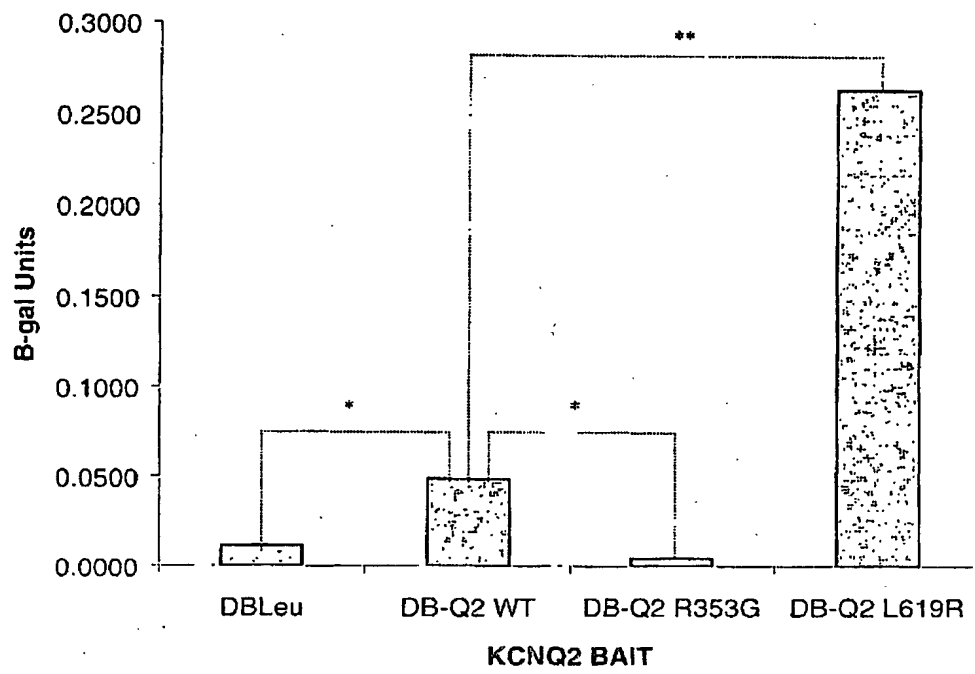


Figure 5





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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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240

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SequencesSSCPre-file7August03.ST25.txt

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gaaggaagta ggctgacata tgaaaagaga ttttcttctc cacaccaggt aaaaatatta  
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SequencesSSCPre-file7August03.ST25.txt

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240

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300

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221

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SequencesSSCPre-file7August03.ST25.txt  
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420

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SequencesSSCPre-file7August03.ST25.txt

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720

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SequencesSSCPre-file7August03.ST25.txt  
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SequencesSSCPre-file7August03.ST25.txt

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1020

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1080

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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2160

cgccagggcc acggcacctc ccccgtaggg gaccacggct ccctggtgcg catcccgccc  
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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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<212> DNA

<213> Homo sapiens

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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1800

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1860

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1920

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1980

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SequencesSSCPre-file7August03.ST25.txt

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480

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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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420

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SequencesSSCPre-file7August03.ST25.txt

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120

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gaggtcggca gaagtgacaa gctttccctg cctggctttg agaacctcac agcaggatat  
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SequencesSSCPre-file7August03.ST25.txt

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SequencesSSCPre-file7August03.ST25.txt

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1980

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2100

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2160

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SequencesSSCPre-file7August03.ST25.txt

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<211> 253

<212> DNA

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<211> 288

<212> DNA

<213> Homo sapiens

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tgatgaactt caagtcgggt aacatatgcc cgccgccctt tccgcatgtg cccgccgccc  
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SequencesSSCPre-file7August03.ST25.txt  
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180

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<213> Homo sapiens

<400> 62  
accggtgatg tggcttggt tagtcatacc ctaaagattg ctcttaagag tgatcttgga

SequencesSSCPre-file7August03.ST25.txt

60

tgcaaatggt catgacagtt tcctagttat tttttcttct tttcttgtag ttactacatc  
120

cagattcctc aagatggatt cctgagcgaa taagcctaca agccccttcc  
170

<210> 63  
<211> 218  
<212> PRT  
<213> Homo sapiens

<400> 63

Met Gly Arg Leu Leu Ala Leu Val Val Gly Ala Ala Leu Val Ser Ser  
1 5 10 15

Ala Cys Gly Gly Cys Val Glu Val Asp Ser Glu Thr Glu Ala Val Tyr  
20 25 30

Gly Met Thr Phe Lys Ile Leu Cys Ile Ser Cys Lys Arg Arg Ser Glu  
35 40 45

Thr Asn Ala Glu Thr Phe Thr Glu Trp Thr Phe Arg Gln Lys Gly Thr  
50 55 60

Glu Glu Phe Val Lys Ile Leu Arg Tyr Glu Asn Glu Val Leu Gln Leu  
65 70 75 80

Glu Glu Asp Glu His Phe Glu Gly Arg Val Val Trp Asn Gly Ser Arg  
85 90 95

Gly Thr Lys Asp Leu Gln Asp Leu Ser Ile Phe Ile Thr Asn Val Thr  
100 105 110

Tyr Asn His Ser Gly Asp Tyr Glu Cys His Val Tyr Arg Leu Leu Phe  
115 120 125

Phe Glu Asn Tyr Glu His Asn Thr Ser Val Val Lys Lys Ile His Ile  
130 135 140

Glu Val Val Asp Lys Ala Asn Arg Asp Met Ala Ser Ile Val Ser Glu  
145 150 155 160

SequencesSSCPre-file7August03.ST25.txt

Ile Met Met Tyr Val Leu Ile Val Val Leu Thr Ile Trp Leu Val Ala  
165 170 175

Glu Met Ile Tyr Cys Tyr Lys Lys Ile Ala Ala Ala Thr Glu Thr Ala  
180 185 190

Ala Gln Glu Asn Ala Ser Glu Tyr Leu Ala Ile Thr Ser Glu Ser Lys  
195 200 205

Glu Asn Cys Thr Gly Val Gln Val Ala Glu  
210 215

<210> 64  
<211> 2005  
<212> PRT  
<213> Homo sapiens

<400> 64

Met Ala Gln Ser Val Leu Val Pro Pro Gly Pro Asp Ser Phe Arg Phe  
1 5 10 15

Phe Thr Arg Glu Ser Leu Ala Ala Ile Glu Gln Arg Ile Ala Glu Glu  
20 25 30

Lys Ala Lys Arg Pro Lys Gln Glu Arg Lys Asp Glu Asp Asp Glu Asn  
35 40 45

Gly Pro Lys Pro Asn Ser Asp Leu Glu Ala Gly Lys Ser Leu Pro Phe  
50 55 60

Ile Tyr Gly Asp Ile Pro Pro Glu Met Val Ser Val Pro Leu Glu Asp  
65 70 75 80

Leu Asp Pro Tyr Tyr Ile Asn Lys Lys Thr Phe Ile Val Leu Asn Lys  
85 90 95

Gly Lys Ala Ile Ser Arg Phe Ser Ala Thr Pro Ala Leu Tyr Ile Leu  
100 105 110

Thr Pro Phe Asn Pro Ile Arg Lys Leu Ala Ile Lys Ile Leu Val His  
115 120 125

SequencesSSCPre-file7August03.ST25.txt

Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val  
130 135 140

Phe Met Thr Met Ser Asn Pro Pro Asp Trp Thr Lys Asn Val Glu Tyr  
145 150 155 160

Thr Phe Thr Gly Ile Tyr Thr Phe Glu Ser Leu Ile Lys Ile Leu Ala  
165 170 175

Arg Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Asp Pro Trp Asn  
180 185 190

Trp Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val  
195 200 205

Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Gln Ala  
210 215 220

Leu Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala  
225 230 235 240

Leu Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val  
245 250 255

Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly  
260 265 270

Asn Leu Arg Asn Lys Cys Leu Gln Trp Pro Pro Asp Asn Ser Ser Phe  
275 280 285

Glu Ile Asn Ile Thr Ser Phe Phe Asn Asn Ser Leu Asp Gly Asn Gly  
290 295 300

Thr Thr Phe Asn Arg Thr Val Ser Ile Phe Asn Trp Asp Glu Tyr Ile  
305 310 315 320

Glu Asp Lys Ser His Phe Tyr Phe Leu Glu Gly Gln Asn Asp Ala Leu  
325 330 335

Leu Cys Gly Asn Ser Ser Asp Ala Gly Gln Cys Pro Glu Gly Tyr Ile  
340 345 350



SequencesSSCPre-file7August03.ST25.txt

Cys Val Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp  
355 360 365

Thr Phe Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp  
370 375 380

Phe Trp Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr  
385 390 395 400

Tyr Met Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu  
405 410 415

Ile Asn Leu Ile Leu Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn  
420 425 430

Gln Ala Thr Leu Glu Glu Ala Glu Gln Lys Glu Ala Glu Phe Gln Gln  
435 440 445

Met Leu Glu Gln Leu Lys Lys Gln Gln Glu Glu Ala Gln Ala Ala Ala  
450 455 460

Ala Ala Ala Ser Ala Glu Ser Arg Asp Phe Ser Gly Ala Gly Gly Ile  
465 470 475 480

Gly Val Phe Ser Glu Ser Ser Ser Val Ala Ser Lys Leu Ser Ser Lys  
485 490 495

Ser Glu Lys Glu Leu Lys Asn Arg Arg Lys Lys Lys Lys Gln Lys Glu  
500 505 510

Gln Ser Gly Glu Glu Glu Lys Asn Asp Arg Val Leu Lys Ser Glu Ser  
515 520 525

Glu Asp Ser Ile Arg Arg Lys Gly Phe Arg Phe Ser Leu Glu Gly Ser  
530 535 540

Arg Leu Thr Tyr Glu Lys Arg Phe Ser Ser Pro His Gln Ser Leu Leu  
545 550 555 560

Ser Ile Arg Gly Ser Leu Phe Ser Pro Arg Arg Asn Ser Arg Ala Ser

Leu Phe Ser Phe Arg Gly Arg Ala Lys Asp Ile Gly Ser Glu Asn Asp  
580 585 590

Phe Ala Asp Asp Glu His Ser Thr Phe Glu Asp Asn Asp Ser Arg Arg  
595 600 605

Asp Ser Leu Phe Val Pro His Arg His Gly Glu Arg Arg His Ser Asn  
610 615 620

Val Ser Gln Ala Ser Arg Ala Ser Arg Val Leu Pro Ile Leu Pro Met  
625 630 635 640

Asn Gly Lys Met His Ser Ala Val Asp Cys Asn Gly Val Val Ser Leu  
645 650 655

Val Gly Gly Pro Ser Thr Leu Thr Ser Ala Gly Gln Leu Leu Pro Glu  
660 665 670

Gly Thr Thr Thr Glu Thr Glu Ile Arg Lys Arg Arg Ser Ser Ser Tyr  
675 680 685

His Val Ser Met Asp Leu Leu Glu Asp Pro Thr Ser Arg Gln Arg Ala  
690 695 700

Met Ser Ile Ala Ser Ile Leu Thr Asn Thr Met Glu Glu Leu Glu Glu  
705 710 715 720

Ser Arg Gln Lys Cys Pro Pro Cys Trp Tyr Lys Phe Ala Asn Met Cys  
725 730 735

Leu Ile Trp Asp Cys Cys Lys Pro Trp Leu Lys Val Lys His Leu Val  
740 745 750

Asn Leu Val Val Met Asp Pro Phe Val Asp Leu Ala Ile Thr Ile Cys  
755 760 765

Ile Val Leu Asn Thr Leu Phe Met Ala Met Glu His Tyr Pro Met Thr  
770 775 780

SequencesSSCPre-file7August03.ST25.txt

Glu Gln Phe Ser Ser Val Leu Ser Val Gly Asn Leu Val Phe Thr Gly  
785 790 795 800

Ile Phe Thr Ala Glu Met Phe Leu Lys Ile Ile Ala Met Asp Pro Tyr  
805 810 815

Tyr Tyr Phe Gln Glu Gly Trp Asn Ile Phe Asp Gly Phe Ile Val Ser  
820 825 830

Leu Ser Leu Met Glu Leu Gly Leu Ala Asn Val Glu Gly Leu Ser Val  
835 840 845

Leu Arg Ser Phe Arg Leu Leu Arg Val Phe Lys Leu Ala Lys Ser Trp  
850 855 860

Pro Thr Leu Asn Met Leu Ile Lys Ile Ile Gly Asn Ser Val Gly Ala  
865 870 875 880

Leu Gly Asn Leu Thr Leu Val Leu Ala Ile Ile Val Phe Ile Phe Ala  
885 890 895

Val Val Gly Met Gln Leu Phe Gly Lys Ser Tyr Lys Glu Cys Val Cys  
900 905 910

Lys Ile Ser Asn Asp Cys Glu Leu Pro Arg Trp His Met His Asp Phe  
915 920 925

Phe His Ser Phe Leu Ile Val Phe Arg Val Leu Cys Gly Glu Trp Ile  
930 935 940

Glu Thr Met Trp Asp Cys Met Glu Val Ala Gly Gln Thr Met Cys Leu  
945 950 955 960

Thr Val Phe Met Met Val Met Val Ile Gly Asn Leu Val Val Leu Asn  
965 970 975

Leu Phe Leu Ala Leu Leu Leu Ser Ser Phe Ser Ser Asp Asn Leu Ala  
980 985 990

Ala Thr Asp Asp Asp Asn Glu Met Asn Asn Leu Gln Ile Ala Val Gly  
995 1000 1005

SequencesSSCPre-file7August03.ST25.txt

Arg Met	Gln Lys Gly Ile Asp	Phe Val Lys Arg Lys	Ile Arg Glu
1010	1015	1020	
Phe Ile	Gln Lys Ala Phe Val	Arg Lys Gln Lys Ala	Leu Asp Glu
1025	1030	1035	
Ile Lys	Pro Leu Glu Asp Leu	Asn Asn Lys Lys Asp	Ser Cys Ile
1040	1045	1050	
Ser Asn	His Thr Thr Ile Glu	Ile Gly Lys Asp Leu	Asn Tyr Leu
1055	1060	1065	
Lys Asp	Gly Asn Gly Thr Thr	Ser Gly Ile Gly Ser	Ser Val Glu
1070	1075	1080	
Lys Tyr	Val Val Asp Glu Ser	Asp Tyr Met Ser Phe	Ile Asn Asn
1085	1090	1095	
Pro Ser	Leu Thr Val Thr Val	Pro Ile Ala Val Gly	Glu Ser Asp
1100	1105	1110	
Phe Glu	Asn Leu Asn Thr Glu	Glu Phe Ser Ser Glu	Ser Asp Met
1115	1120	1125	
Glu Glu	Ser Lys Glu Lys Leu	Asn Ala Thr Ser Ser	Ser Glu Gly
1130	1135	1140	
Ser Thr	Val Asp Ile Gly Ala	Pro Ala Glu Gly Glu	Gln Pro Glu
1145	1150	1155	
Val Glu	Pro Glu Glu Ser Leu	Glu Pro Glu Ala Cys	Phe Thr Glu
1160	1165	1170	
Asp Cys	Val Arg Lys Phe Lys	Cys Cys Gln Ile Ser	Ile Glu Glu
1175	1180	1185	
Gly Lys	Gly Lys Leu Trp Trp	Asn Leu Arg Lys Thr	Cys Tyr Lys
1190	1195	1200	
Ile Val	Glu His Asn Trp Phe	Glu Thr Phe Ile Val	Phe Met Ile
1205	1210	1215	

SequencesSSCPre-file7August03.ST25.txt

Leu Leu Ser Ser Gly Ala Leu Ala Phe Glu Asp Ile Tyr Ile Glu  
1220 1225 1230

Gln Arg Lys Thr Ile Lys Thr Met Leu Glu Tyr Ala Asp Lys Val  
1235 1240 1245

Phe Thr Tyr Ile Phe Ile Leu Glu Met Leu Leu Lys Trp Val Ala  
1250 1255 1260

Tyr Gly Phe Gln Val Tyr Phe Thr Asn Ala Trp Cys Trp Leu Asp  
1265 1270 1275

Phe Leu Ile Val Asp Val Ser Leu Val Ser Leu Thr Ala Asn Ala  
1280 1285 1290

Leu Gly Tyr Ser Glu Leu Gly Ala Ile Lys Ser Leu Arg Thr Leu  
1295 1300 1305

Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser Arg Phe Glu Gly Met  
1310 1315 1320

Arg Val Val Val Asn Ala Leu Leu Gly Ala Ile Pro Ser Ile Met  
1325 1330 1335

Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Ile Phe Ser Ile  
1340 1345 1350

Met Gly Val Asn Leu Phe Ala Gly Lys Phe Tyr His Cys Ile Asn  
1355 1360 1365

Tyr Thr Thr Gly Glu Met Phe Asp Val Ser Val Val Asn Asn Tyr  
1370 1375 1380

Ser Glu Cys Lys Ala Leu Ile Glu Ser Asn Gln Thr Ala Arg Trp  
1385 1390 1395

Lys Asn Val Lys Val Asn Phe Asp Asn Val Gly Leu Gly Tyr Leu  
1400 1405 1410

Ser Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp Ile Met

1425

Met Phe Leu Ala Glu Leu Ile Glu Lys Tyr Phe Val Ser Pro Thr  
1610 1615 1620

SequencesSSCPre-file7August03.ST25.txt

Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg  
1625 1630 1635

Leu Ile Lys Gly Ala Lys Gly Ile Arg Thr Leu Leu Phe Ala Leu  
1640 1645 1650

Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu Phe  
1655 1660 1665

Leu Val Met Phe Ile Tyr Ala Ile Phe Gly Met Ser Asn Phe Ala  
1670 1675 1680

Tyr Val Lys Arg Glu Val Gly Ile Asp Asp Met Phe Asn Phe Glu  
1685 1690 1695

Thr Phe Gly Asn Ser Met Ile Cys Leu Phe Gln Ile Thr Thr Ser  
1700 1705 1710

Ala Gly Trp Asp Gly Leu Leu Ala Pro Ile Leu Asn Ser Gly Pro  
1715 1720 1725

Pro Asp Cys Asp Pro Asp Lys Asp His Pro Gly Ser Ser Val Lys  
1730 1735 1740

Gly Asp Cys Gly Asn Pro Ser Val Gly Ile Phe Phe Phe Val Ser  
1745 1750 1755

Tyr Ile Ile Ile Ser Phe Leu Val Val Leu Asn Met Tyr Ile Ala  
1760 1765 1770

Val Ile Leu Glu Asn Phe Ser Val Ala Thr Glu Glu Ser Ala Glu  
1775 1780 1785

Pro Leu Ser Glu Asp Asp Phe Glu Met Phe Tyr Glu Val Trp Glu  
1790 1795 1800

Lys Phe Asp Pro Asp Ala Thr Gln Phe Ile Glu Phe Ala Lys Leu  
1805 1810 1815

Ser Asp Phe Ala Asp Ala Leu Asp Pro Pro Leu Leu Ile Ala Lys  
1820 1825 1830

SequencesSSCPre-file7August03.ST25.txt

Pro Asn Lys Val Gln Leu Ile Ala Met Asp Leu Pro Met Val Ser  
1835 1840 1845

Gly Asp Arg Ile His Cys Leu Asp Ile Leu Phe Ala Phe Thr Lys  
1850 1855 1860

Arg Val Leu Gly Glu Ser Gly Glu Met Asp Ala Leu Arg Ile Gln  
1865 1870 1875

Met Glu Glu Arg Phe Met Ala Ser Asn Pro Ser Lys Val Ser Tyr  
1880 1885 1890

Glu Pro Ile Thr Thr Thr Leu Lys Arg Lys Gln Glu Glu Val Ser  
1895 1900 1905

Ala Ile Ile Ile Gln Arg Ala Tyr Arg Arg Tyr Leu Leu Lys Gln  
1910 1915 1920

Lys Val Lys Lys Val Ser Ser Ile Tyr Lys Lys Asp Lys Gly Lys  
1925 1930 1935

Glu Cys Asp Gly Thr Pro Ile Lys Glu Asp Thr Leu Ile Asp Lys  
1940 1945 1950

Leu Asn Glu Asn Ser Thr Pro Glu Lys Thr Asp Met Thr Pro Ser  
1955 1960 1965

Thr Thr Ser Pro Pro Ser Tyr Asp Ser Val Thr Lys Pro Glu Lys  
1970 1975 1980

Glu Lys Phe Glu Lys Asp Lys Ser Glu Lys Glu Asp Lys Gly Lys  
1985 1990 1995

Asp Ile Arg Glu Ser Lys Lys  
2000 2005

<210> 65  
<211> 2005  
<212> PRT  
<213> Homo sapiens  
<400> 65



SequencesSSCPre-file7August03.ST25.txt

Met Ala Gln Ser Val Leu Val Pro Pro Gly Pro Asp Ser Phe Arg Phe  
1 5 10 15

Phe Thr Arg Glu Ser Leu Ala Ala Ile Glu Gln Arg Ile Ala Glu Glu  
20 25 30

Lys Ala Lys Arg Pro Lys Gln Glu Arg Lys Asp Glu Asp Asp Glu Asn  
35 40 45

Gly Pro Lys Pro Asn Ser Asp Leu Glu Ala Gly Lys Ser Leu Pro Phe  
50 55 60

Ile Tyr Gly Asp Ile Pro Pro Glu Met Val Ser Val Pro Leu Glu Asp  
65 70 75 80

Leu Asp Pro Tyr Tyr Ile Asn Lys Lys Thr Phe Ile Val Leu Asn Lys  
85 90 95

Gly Lys Ala Ile Ser Arg Phe Ser Ala Thr Pro Ala Leu Tyr Ile Leu  
100 105 110

Thr Pro Phe Asn Pro Ile Arg Lys Leu Ala Ile Lys Ile Leu Val His  
115 120 125

Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val  
130 135 140

Phe Met Thr Met Ser Asn Pro Pro Asp Trp Thr Lys Asn Val Glu Tyr  
145 150 155 160

Thr Phe Thr Gly Ile Tyr Thr Phe Glu Ser Leu Ile Lys Ile Leu Ala  
165 170 175

Arg Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Asp Pro Trp Asn  
180 185 190

Trp Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val  
195 200 205

Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg Ala  
210 215 220

SequencesSSCPre-file7August03.ST25.txt

Leu Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala  
 225 230 235 240

Leu Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val  
 245 250 255

Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly  
 260 265 270

Asn Leu Arg Asn Lys Cys Leu Gln Trp Pro Pro Asp Asn Ser Ser Phe  
 275 280 285

Glu Ile Asn Ile Thr Ser Phe Phe Asn Asn Ser Leu Asp Gly Asn Gly  
 290 295 300

Thr Thr Phe Asn Arg Thr Val Ser Ile Phe Asn Trp Asp Glu Tyr Ile  
 305 310 315 320

Glu Asp Lys Ser His Phe Tyr Phe Leu Glu Gly Gln Asn Asp Ala Leu  
 325 330 335

Leu Cys Gly Asn Ser Ser Asp Ala Gly Gln Cys Pro Glu Gly Tyr Ile  
 340 345 350

Cys Val Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp  
 355 360 365

Thr Phe Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp  
 370 375 380

Phe Trp Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr  
 385 390 395 400

Tyr Met Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu  
 405 410 415

Ile Asn Leu Ile Leu Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn  
 420 425 430

Gln Ala Thr Leu Glu Glu Ala Glu Gln Lys Glu Ala Glu Phe Gln Gln

SequencesSSCPre-file7August03.ST25.txt

435

440

445

Met Leu Glu Gln Leu Lys Lys Gln Gln Glu Glu Ala Gln Ala Ala Ala  
450 455 460

Ala Ala Ala Ser Ala Glu Ser Arg Asp Phe Ser Gly Ala Gly Gly Ile  
465 470 475 480

Gly Val Phe Ser Glu Ser Ser Ser Val Ala Ser Lys Leu Ser Ser Lys  
485 490 495

Ser Glu Lys Glu Leu Lys Asn Arg Arg Lys Lys Lys Lys Gln Lys Glu  
500 505 510

Gln Ser Gly Glu Glu Glu Lys Asn Asp Arg Val Leu Lys Ser Glu Ser  
515 520 525

Glu Asp Ser Ile Arg Arg Lys Gly Phe Arg Phe Ser Leu Glu Gly Ser  
530 535 540

Arg Leu Thr Tyr Glu Lys Arg Phe Ser Ser Pro His Gln Ser Leu Leu  
545 550 555 560

Ser Ile Arg Gly Ser Leu Phe Ser Pro Arg Arg Asn Ser Arg Ala Ser  
565 570 575

Leu Phe Ser Phe Arg Gly Arg Ala Lys Asp Ile Gly Ser Glu Asn Asp  
580 585 590

Phe Ala Asp Asp Glu His Ser Thr Phe Glu Asp Asn Asp Ser Arg Arg  
595 600 605

Asp Ser Leu Phe Val Pro His Arg His Gly Glu Arg Arg His Ser Asn  
610 615 620

Val Ser Gln Ala Ser Arg Ala Ser Arg Val Leu Pro Ile Leu Pro Met  
625 630 635 640

Asn Gly Lys Met His Ser Ala Val Asp Cys Asn Gly Val Val Ser Leu  
645 650 655

SequencesSSCPre-file7August03.ST25.txt

Val Gly Gly Pro Ser Thr Leu Thr Ser Ala Gly Gln Leu Leu Pro Glu  
660 665 670

Gly Thr Thr Thr Glu Thr Glu Ile Arg Lys Arg Arg Ser Ser Ser Tyr  
675 680 685

His Val Ser Met Asp Leu Leu Glu Asp Pro Thr Ser Arg Gln Arg Ala  
690 695 700

Met Ser Ile Ala Ser Ile Leu Thr Asn Thr Met Glu Glu Leu Glu Glu  
705 710 715 720

Ser Arg Gln Lys Cys Pro Pro Cys Trp Tyr Lys Phe Ala Asn Met Cys  
725 730 735

Leu Ile Trp Asp Cys Cys Lys Pro Trp Leu Lys Val Lys His Leu Val  
740 745 750

Asn Leu Val Val Met Asp Pro Phe Val Asp Leu Ala Ile Thr Ile Cys  
755 760 765

Ile Val Leu Asn Thr Leu Phe Met Ala Met Glu His Tyr Pro Met Thr  
770 775 780

Glu Gln Phe Ser Ser Val Leu Ser Val Gly Asn Leu Val Phe Thr Gly  
785 790 795 800

Ile Phe Thr Ala Glu Met Phe Leu Lys Ile Ile Ala Met Asp Pro Tyr  
805 810 815

Tyr Tyr Phe Gln Glu Gly Trp Asn Ile Phe Asp Gly Phe Ile Val Ser  
820 825 830

Leu Ser Leu Met Glu Leu Gly Leu Ala Asn Val Glu Gly Leu Ser Val  
835 840 845

Leu Arg Ser Phe Arg Leu Leu Arg Val Phe Lys Leu Ala Lys Ser Trp  
850 855 860

Pro Thr Leu Asn Met Leu Ile Lys Ile Ile Gly Asn Ser Val Gly Ala  
865 870 875 880

SequencesSSCPre-file7August03.ST25.txt

Leu Gly Asn Leu Thr Leu Val Leu Ala Ile Ile Ile Phe Ile Phe Ala  
885 890 895

Val Val Gly Met Gln Leu Phe Gly Lys Ser Tyr Lys Glu Cys Val Cys  
900 905 910

Lys Ile Ser Asn Asp Cys Glu Leu Pro Arg Trp His Met His Asp Phe  
915 920 925

Phe His Ser Phe Leu Ile Val Phe Arg Val Leu Cys Gly Glu Trp Ile  
930 935 940

Glu Thr Met Trp Asp Cys Met Glu Val Ala Gly Gln Thr Met Cys Leu  
945 950 955 960

Thr Val Phe Met Met Val Met Val Ile Gly Asn Leu Val Val Leu Asn  
965 970 975

Leu Phe Leu Ala Leu Leu Leu Ser Ser Phe Ser Ser Asp Asn Leu Ala  
980 985 990

Ala Thr Asp Asp Asp Asn Glu Met Asn Asn Leu Gln Ile Ala Val Gly  
995 1000 1005

Arg Met Gln Lys Gly Ile Asp Phe Val Lys Arg Lys Ile Arg Glu  
1010 1015 1020

Phe Ile Gln Lys Ala Phe Val Arg Lys Gln Lys Ala Leu Asp Glu  
1025 1030 1035

Ile Lys Pro Leu Glu Asp Leu Asn Asn Lys Lys Asp Ser Cys Ile  
1040 1045 1050

Ser Asn His Thr Thr Ile Glu Ile Gly Lys Asp Leu Asn Tyr Leu  
1055 1060 1065

Lys Asp Gly Asn Gly Thr Thr Ser Gly Ile Gly Ser Ser Val Glu  
1070 1075 1080

Lys Tyr Val Val Asp Glu Ser Asp Tyr Met Ser Phe Ile Asn Asn  
1085 1090 1095

SequencesSSCPre-file7August03.ST25.txt

Pro Ser Leu Thr Val Thr Val Pro Ile Ala Val Gly Glu Ser Asp  
1100 1105 1110

Phe Glu Asn Leu Asn Thr Glu Glu Phe Ser Ser Glu Ser Asp Met  
1115 1120 1125

Glu Glu Ser Lys Glu Lys Leu Asn Ala Thr Ser Ser Ser Glu Gly  
1130 1135 1140

Ser Thr Val Asp Ile Gly Ala Pro Ala Glu Gly Glu Gln Pro Glu  
1145 1150 1155

Val Glu Pro Glu Glu Ser Leu Glu Pro Glu Ala Cys Phe Thr Glu  
1160 1165 1170

Asp Cys Val Arg Lys Phe Lys Cys Cys Gln Ile Ser Ile Glu Glu  
1175 1180 1185

Gly Lys Gly Lys Leu Trp Trp Asn Leu Arg Lys Thr Cys Tyr Lys  
1190 1195 1200

Ile Val Glu His Asn Trp Phe Glu Thr Phe Ile Val Phe Met Ile  
1205 1210 1215

Leu Leu Ser Ser Gly Ala Leu Ala Phe Glu Asp Ile Tyr Ile Glu  
1220 1225 1230

Gln Arg Lys Thr Ile Lys Thr Met Leu Glu Tyr Ala Asp Lys Val  
1235 1240 1245

Phe Thr Tyr Ile Phe Ile Leu Glu Met Leu Leu Lys Trp Val Ala  
1250 1255 1260

Tyr Gly Phe Gln Val Tyr Phe Thr Asn Ala Trp Cys Trp Leu Asp  
1265 1270 1275

Phe Leu Ile Val Asp Val Ser Leu Val Ser Leu Thr Ala Asn Ala  
1280 1285 1290

Leu Gly Tyr Ser Glu Leu Gly Ala Ile Lys Ser Leu Arg Thr Leu

## SequencesSSCPre-file7August03.ST25.txt

1295

1300

1305

Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser Arg Phe Glu Gly Met  
 1310 1315 1320

Arg Val Val Val Asn Ala Leu Leu Gly Ala Ile Pro Ser Ile Met  
 1325 1330 1335

Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Ile Phe Ser Ile  
 1340 1345 1350

Met Gly Val Asn Leu Phe Ala Gly Lys Phe Tyr His Cys Ile Asn  
 1355 1360 1365

Tyr Thr Thr Gly Glu Met Phe Asp Val Ser Val Val Asn Asn Tyr  
 1370 1375 1380

Ser Glu Cys Lys Ala Leu Ile Glu Ser Asn Gln Thr Ala Arg Trp  
 1385 1390 1395

Lys Asn Val Lys Val Asn Phe Asp Asn Val Gly Leu Gly Tyr Leu  
 1400 1405 1410

Ser Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp Ile Met  
 1415 1420 1425

Tyr Ala Ala Val Asp Ser Arg Asn Val Glu Leu Gln Pro Lys Tyr  
 1430 1435 1440

Glu Asp Asn Leu Tyr Met Tyr Leu Tyr Phe Val Ile Phe Ile Ile  
 1445 1450 1455

Phe Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile Gly Val Ile Ile  
 1460 1465 1470

Asp Asn Phe Asn Gln Gln Lys Lys Lys Phe Gly Gly Gln Asp Ile  
 1475 1480 1485

Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys  
 1490 1495 1500

SequencesSSCPre-file7August03.ST25.txt

Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro Ala Asn  
1505 1510 1515

Lys Phe Gln Gly Met Val Phe Asp Phe Val Thr Lys Gln Val Phe  
1520 1525 1530

Asp Ile Ser Ile Met Ile Leu Ile Cys Leu Asn Met Val Thr Met  
1535 1540 1545

Met Val Glu Thr Asp Asp Gln Ser Gln Glu Met Thr Asn Ile Leu  
1550 1555 1560

Tyr Trp Ile Asn Leu Val Phe Ile Val Leu Phe Thr Gly Glu Cys  
1565 1570 1575

Val Leu Lys Leu Ile Ser Leu Arg Tyr Tyr Tyr Phe Thr Ile Gly  
1580 1585 1590

Trp Asn Ile Phe Asp Phe Val Val Val Ile Leu Ser Ile Val Gly  
1595 1600 1605

Met Phe Leu Ala Glu Leu Ile Glu Lys Tyr Phe Val Ser Pro Thr  
1610 1615 1620

Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg  
1625 1630 1635

Leu Ile Lys Gly Ala Lys Gly Ile Arg Thr Leu Leu Phe Ala Leu  
1640 1645 1650

Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu Phe  
1655 1660 1665

Leu Val Met Phe Ile Tyr Ala Ile Phe Gly Met Ser Asn Phe Ala  
1670 1675 1680

Tyr Val Lys Arg Glu Val Gly Ile Asp Asp Met Phe Asn Phe Glu  
1685 1690 1695

Thr Phe Gly Asn Ser Met Ile Cys Leu Phe Gln Ile Thr Thr Ser  
1700 1705 1710



SequencesSSCPre-file7August03.ST25.txt

Ala Gly Trp Asp Gly Leu Leu Ala Pro Ile Leu Asn Ser Gly Pro  
1715 1720 1725

Pro Asp Cys Asp Pro Asp Lys Asp His Pro Gly Ser Ser Val Lys  
1730 1735 1740

Gly Asp Cys Gly Asn Pro Ser Val Gly Ile Phe Phe Phe Val Ser  
1745 1750 1755

Tyr Ile Ile Ile Ser Phe Leu Val Val Leu Asn Met Tyr Ile Ala  
1760 1765 1770

Val Ile Leu Glu Asn Phe Ser Val Ala Thr Glu Glu Ser Ala Glu  
1775 1780 1785

Pro Leu Ser Glu Asp Asp Phe Glu Met Phe Tyr Glu Val Trp Glu  
1790 1795 1800

Lys Phe Asp Pro Asp Ala Thr Gln Phe Ile Glu Phe Ala Lys Leu  
1805 1810 1815

Ser Asp Phe Ala Asp Ala Leu Asp Pro Pro Leu Leu Ile Ala Lys  
1820 1825 1830

Pro Asn Lys Val Gln Leu Ile Ala Met Asp Leu Pro Met Val Ser  
1835 1840 1845

Gly Asp Arg Ile His Cys Leu Asp Ile Leu Phe Ala Phe Thr Lys  
1850 1855 1860

Arg Val Leu Gly Glu Ser Gly Glu Met Asp Ala Leu Arg Ile Gln  
1865 1870 1875

Met Glu Glu Arg Phe Met Ala Ser Asn Pro Ser Lys Val Ser Tyr  
1880 1885 1890

Glu Pro Ile Thr Thr Thr Leu Lys Arg Lys Gln Glu Glu Val Ser  
1895 1900 1905

Ala Ile Ile Ile Gln Arg Ala Tyr Arg Arg Tyr Leu Leu Lys Gln  
1910 1915 1920

SequencesSSCPre-file7August03.ST25.txt

Lys Val Lys Lys Val Ser Ser Ile Tyr Lys Lys Asp Lys Gly Lys  
1925 1930 1935

Glu Cys Asp Gly Thr Pro Ile Lys Glu Asp Thr Leu Ile Asp Lys  
1940 1945 1950

Leu Asn Glu Asn Ser Thr Pro Glu Lys Thr Asp Met Thr Pro Ser  
1955 1960 1965

Thr Thr Ser Pro Pro Ser Tyr Asp Ser Val Thr Lys Pro Glu Lys  
1970 1975 1980

Glu Lys Phe Glu Lys Asp Lys Ser Glu Lys Glu Asp Lys Gly Lys  
1985 1990 1995

Asp Ile Arg Glu Ser Lys Lys  
2000 2005

<210> 66  
<211> 2005  
<212> PRT  
<213> Homo sapiens

<400> 66

Met Ala Gln Ser Val Leu Val Pro Pro Gly Pro Asp Ser Phe Arg Phe  
1 5 10 15

Phe Thr Arg Glu Ser Leu Ala Ala Ile Glu Gln Arg Ile Ala Glu Glu  
20 25 30

Lys Ala Lys Arg Pro Lys Gln Glu Arg Lys Asp Glu Asp Asp Glu Asn  
35 40 45

Gly Pro Lys Pro Asn Ser Asp Leu Glu Ala Gly Lys Ser Leu Pro Phe  
50 55 60

Ile Tyr Gly Asp Ile Pro Pro Glu Met Val Ser Val Pro Leu Glu Asp  
65 70 75 80

Leu Asp Pro Tyr Tyr Ile Asn Lys Lys Thr Phe Ile Val Leu Asn Lys  
85 90 95

SequencesSSCPre-file7August03.ST25.txt

Gly Lys Ala Ile Ser Arg Phe Ser Ala Thr Pro Ala Leu Tyr Ile Leu  
100 105 110

Thr Pro Phe Asn Pro Ile Arg Lys Leu Ala Ile Lys Ile Leu Val His  
115 120 125

Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val  
130 135 140

Phe Met Thr Met Ser Asn Pro Pro Asp Trp Thr Lys Asn Val Glu Tyr  
145 150 155 160

Thr Phe Thr Gly Ile Tyr Thr Phe Glu Ser Leu Ile Lys Ile Leu Ala  
165 170 175

Arg Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Asp Pro Trp Asn  
180 185 190

Trp Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val  
195 200 205

Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg Ala  
210 215 220

Leu Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala  
225 230 235 240

Leu Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val  
245 250 255

Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly  
260 265 270

Asn Leu Arg Asn Lys Cys Leu Gln Trp Pro Pro Asp Asn Ser Ser Phe  
275 280 285

Glu Ile Asn Ile Thr Ser Phe Phe Asn Asn Ser Leu Asp Gly Asn Gly  
290 295 300

Thr Thr Phe Asn Arg Thr Val Ser Ile Phe Asn Trp Asp Glu Tyr Ile

## SequencesSSCPre-file7August03.ST25.txt

305

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Glu Asp Lys Ser His Phe Tyr Phe Leu Glu Gly Gln Asn Asp Ala Leu  
 325 330 335

Leu Cys Gly Asn Ser Ser Asp Ala Gly Gln Cys Pro Glu Gly Tyr Ile  
 340 345 350

Cys Val Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp  
 355 360 365

Thr Phe Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp  
 370 375 380

Phe Trp Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr  
 385 390 395 400

Tyr Met Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu  
 405 410 415

Ile Asn Leu Ile Leu Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn  
 420 425 430

Gln Ala Thr Leu Glu Glu Ala Glu Gln Lys Glu Ala Glu Phe Gln Gln  
 435 440 445

Met Leu Glu Gln Leu Lys Lys Gln Gln Glu Glu Ala Gln Ala Ala Ala  
 450 455 460

Ala Ala Ala Ser Ala Glu Ser Arg Asp Phe Ser Gly Ala Gly Gly Ile  
 465 470 475 480

Gly Val Phe Ser Glu Ser Ser Ser Val Ala Ser Lys Leu Ser Ser Lys  
 485 490 495

Ser Glu Lys Glu Leu Lys Asn Arg Arg Lys Lys Lys Lys Gln Lys Glu  
 500 505 510

Gln Ser Gly Glu Glu Glu Lys Asn Asp Arg Val Leu Lys Ser Glu Ser  
 515 520 525

SequencesSSCPre-file7August03.ST25.txt

Glu Asp Ser Ile Arg Arg Lys Gly Phe Arg Phe Ser Leu Glu Gly Ser  
530 535 540

Arg Leu Thr Tyr Glu Lys Arg Phe Ser Ser Pro His Gln Ser Leu Leu  
545 550 555 560

Ser Ile Arg Gly Ser Leu Phe Ser Pro Arg Arg Asn Ser Arg Ala Ser  
565 570 575

Leu Phe Ser Phe Arg Gly Arg Ala Lys Asp Ile Gly Ser Glu Asn Asp  
580 585 590

Phe Ala Asp Asp Glu His Ser Thr Phe Glu Asp Asn Asp Ser Arg Arg  
595 600 605

Asp Ser Leu Phe Val Pro His Arg His Gly Glu Arg Arg His Ser Asn  
610 615 620

Val Ser Gln Ala Ser Arg Ala Ser Arg Val Leu Pro Ile Leu Pro Met  
625 630 635 640

Asn Gly Lys Met His Ser Ala Val Asp Cys Asn Gly Val Val Ser Leu  
645 650 655

Val Gly Gly Pro Ser Thr Leu Thr Ser Ala Gly Gln Leu Leu Pro Glu  
660 665 670

Gly Thr Thr Thr Glu Thr Glu Ile Arg Lys Arg Arg Ser Ser Ser Tyr  
675 680 685

His Val Ser Met Asp Leu Leu Glu Asp Pro Thr Ser Arg Gln Arg Ala  
690 695 700

Met Ser Ile Ala Ser Ile Leu Thr Asn Thr Met Glu Glu Leu Glu Glu  
705 710 715 720

Ser Arg Gln Lys Cys Pro Pro Cys Trp Tyr Lys Phe Ala Asn Met Cys  
725 730 735

Leu Ile Trp Asp Cys Cys Lys Pro Trp Leu Lys Val Lys His Leu Val  
740 745 750

SequencesSSCPre-file7August03.ST25.txt

Asn Leu Val Val Met Asp Pro Phe Val Asp Leu Ala Ile Thr Ile Cys  
755 760 765

Ile Val Leu Asn Thr Leu Phe Met Ala Met Glu His Tyr Pro Met Thr  
770 775 780

Glu Gln Phe Ser Ser Val Leu Ser Val Gly Asn Leu Val Phe Thr Gly  
785 790 795 800

Ile Phe Thr Ala Glu Met Phe Leu Lys Ile Ile Ala Met Asp Pro Tyr  
805 810 815

Tyr Tyr Phe Gln Glu Gly Trp Asn Ile Phe Asp Gly Phe Ile Val Ser  
820 825 830

Leu Ser Leu Met Glu Leu Gly Leu Ala Asn Val Glu Gly Leu Ser Val  
835 840 845

Leu Arg Ser Phe Arg Leu Leu Arg Val Phe Lys Leu Ala Lys Ser Trp  
850 855 860

Pro Thr Leu Asn Met Leu Ile Lys Ile Ile Gly Asn Ser Val Gly Ala  
865 870 875 880

Leu Gly Asn Leu Thr Leu Val Leu Ala Ile Ile Val Phe Ile Phe Ala  
885 890 895

Val Val Gly Met Gln Leu Phe Gly Lys Ser Tyr Lys Glu Cys Val Cys  
900 905 910

Lys Ile Ser Asn Asp Cys Glu Leu Pro Arg Trp His Met His Asp Phe  
915 920 925

Phe His Ser Phe Leu Ile Val Phe Arg Val Leu Cys Gly Glu Trp Ile  
930 935 940

Glu Thr Met Trp Asp Cys Met Glu Val Ala Gly Gln Thr Met Cys Leu  
945 950 955 960

Thr Val Phe Met Met Val Met Val Ile Gly Asn Leu Val Val Leu Asn  
965 970 975

SequencesSSCPre-file7August03.ST25.txt

Leu Phe Leu Ala Leu Leu Leu Ser Ser Phe Ser Ser Asp Asn Leu Ala  
980 985 990

Ala Thr Asp Asp Asp Asn Glu Met Asn Asn Ile Gln Ile Ala Val Gly  
995 1000 1005

Arg Met Gln Lys Gly Ile Asp Phe Val Lys Arg Lys Ile Arg Glu  
1010 1015 1020

Phe Ile Gln Lys Ala Phe Val Arg Lys Gln Lys Ala Leu Asp Glu  
1025 1030 1035

Ile Lys Pro Leu Glu Asp Leu Asn Asn Lys Lys Asp Ser Cys Ile  
1040 1045 1050

Ser Asn His Thr Thr Ile Glu Ile Gly Lys Asp Leu Asn Tyr Leu  
1055 1060 1065

Lys Asp Gly Asn Gly Thr Thr Ser Gly Ile Gly Ser Ser Val Glu  
1070 1075 1080

Lys Tyr Val Val Asp Glu Ser Asp Tyr Met Ser Phe Ile Asn Asn  
1085 1090 1095

Pro Ser Leu Thr Val Thr Val Pro Ile Ala Val Gly Glu Ser Asp  
1100 1105 1110

Phe Glu Asn Leu Asn Thr Glu Glu Phe Ser Ser Glu Ser Asp Met  
1115 1120 1125

Glu Glu Ser Lys Glu Lys Leu Asn Ala Thr Ser Ser Ser Glu Gly  
1130 1135 1140

Ser Thr Val Asp Ile Gly Ala Pro Ala Glu Gly Glu Gln Pro Glu  
1145 1150 1155

Val Glu Pro Glu Glu Ser Leu Glu Pro Glu Ala Cys Phe Thr Glu  
1160 1165 1170

Asp Cys Val Arg Lys Phe Lys Cys Cys Gln Ile Ser Ile Glu Glu

## 1175

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SequencesSSCPre-file7August03.ST25.txt

Ser	Glu	Cys	Lys	Ala	Leu	Ile	Glu	Ser	Asn	Gln	Thr	Ala	Arg	Trp
1385						1390					1395			
Lys	Asn	Val	Lys	Val	Asn	Phe	Asp	Asn	Val	Gly	Leu	Gly	Tyr	Leu
1400						1405					1410			
Ser	Leu	Leu	Gln	Val	Ala	Thr	Phe	Lys	Gly	Trp	Met	Asp	Ile	Met
1415						1420					1425			
Tyr	Ala	Ala	Val	Asp	Ser	Arg	Asn	Val	Glu	Leu	Gln	Pro	Lys	Tyr
1430						1435					1440			
Glu	Asp	Asn	Leu	Tyr	Met	Tyr	Leu	Tyr	Phe	Val	Ile	Phe	Ile	Ile
1445						1450					1455			
Phe	Gly	Ser	Phe	Phe	Thr	Leu	Asn	Leu	Phe	Ile	Gly	Val	Ile	Ile
1460						1465					1470			
Asp	Asn	Phe	Asn	Gln	Gln	Lys	Lys	Lys	Phe	Gly	Gly	Gln	Asp	Ile
1475						1480					1485			
Phe	Met	Thr	Glu	Glu	Gln	Lys	Lys	Tyr	Tyr	Asn	Ala	Met	Lys	Lys
1490						1495					1500			
Leu	Gly	Ser	Lys	Lys	Pro	Gln	Lys	Pro	Ile	Pro	Arg	Pro	Ala	Asn
1505						1510					1515			
Lys	Phe	Gln	Gly	Met	Val	Phe	Asp	Phe	Val	Thr	Lys	Gln	Val	Phe
1520						1525					1530			
Asp	Ile	Ser	Ile	Met	Ile	Leu	Ile	Cys	Leu	Asn	Met	Val	Thr	Met
1535						1540					1545			
Met	Val	Glu	Thr	Asp	Asp	Gln	Ser	Gln	Glu	Met	Thr	Asn	Ile	Leu
1550						1555					1560			
Tyr	Trp	Ile	Asn	Leu	Val	Phe	Ile	Val	Leu	Phe	Thr	Gly	Glu	Cys
1565						1570					1575			
Val	Leu	Lys	Leu	Ile	Ser	Leu	Arg	Tyr	Tyr	Tyr	Phe	Thr	Ile	Gly
1580						1585					1590			

SequencesSSCPre-file7August03.ST25.txt

Trp	Asn	Ile	Phe	Asp	Phe	Val	Val	Val	Ile	Leu	Ser	Ile	Val	Gly
1595						1600					1605			
Met	Phe	Leu	Ala	Glu	Leu	Ile	Glu	Lys	Tyr	Phe	Val	Ser	Pro	Thr
1610						1615					1620			
Leu	Phe	Arg	Val	Ile	Arg	Leu	Ala	Arg	Ile	Gly	Arg	Ile	Leu	Arg
1625						1630					1635			
Leu	Ile	Lys	Gly	Ala	Lys	Gly	Ile	Arg	Thr	Leu	Leu	Phe	Ala	Leu
1640						1645					1650			
Met	Met	Ser	Leu	Pro	Ala	Leu	Phe	Asn	Ile	Gly	Leu	Leu	Leu	Phe
1655						1660					1665			
Leu	Val	Met	Phe	Ile	Tyr	Ala	Ile	Phe	Gly	Met	Ser	Asn	Phe	Ala
1670						1675					1680			
Tyr	Val	Lys	Arg	Glu	Val	Gly	Ile	Asp	Asp	Met	Phe	Asn	Phe	Glu
1685						1690					1695			
Thr	Phe	Gly	Asn	Ser	Met	Ile	Cys	Leu	Phe	Gln	Ile	Thr	Thr	Ser
1700						1705					1710			
Ala	Gly	Trp	Asp	Gly	Leu	Leu	Ala	Pro	Ile	Leu	Asn	Ser	Gly	Pro
1715						1720					1725			
Pro	Asp	Cys	Asp	Pro	Asp	Lys	Asp	His	Pro	Gly	Ser	Ser	Val	Lys
1730						1735					1740			
Gly	Asp	Cys	Gly	Asn	Pro	Ser	Val	Gly	Ile	Phe	Phe	Phe	Val	Ser
1745						1750					1755			
Tyr	Ile	Ile	Ile	Ser	Phe	Leu	Val	Val	Leu	Asn	Met	Tyr	Ile	Ala
1760						1765					1770			
Val	Ile	Leu	Glu	Asn	Phe	Ser	Val	Ala	Thr	Glu	Glu	Ser	Ala	Glu
1775						1780					1785			
Pro	Leu	Ser	Glu	Asp	Asp	Phe	Glu	Met	Phe	Tyr	Glu	Val	Trp	Glu
1790						1795					1800			

SequencesSSCPre-file7August03.ST25.txt

Lys	Phe	Asp	Pro	Asp	Ala	Thr	Gln	Phe	Ile	Glu	Phe	Ala	Lys	Leu
1805						1810					1815			
Ser	Asp	Phe	Ala	Asp	Ala	Leu	Asp	Pro	Pro	Leu	Leu	Ile	Ala	Lys
1820						1825					1830			
Pro	Asn	Lys	Val	Gln	Leu	Ile	Ala	Met	Asp	Leu	Pro	Met	Val	Ser
1835						1840					1845			
Gly	Asp	Arg	Ile	His	Cys	Leu	Asp	Ile	Leu	Phe	Ala	Phe	Thr	Lys
1850						1855					1860			
Arg	Val	Leu	Gly	Glu	Ser	Gly	Glu	Met	Asp	Ala	Leu	Arg	Ile	Gln
1865						1870					1875			
Met	Glu	Glu	Arg	Phe	Met	Ala	Ser	Asn	Pro	Ser	Lys	Val	Ser	Tyr
1880						1885					1890			
Glu	Pro	Ile	Thr	Thr	Thr	Leu	Lys	Arg	Lys	Gln	Glu	Glu	Val	Ser
1895						1900					1905			
Ala	Ile	Ile	Ile	Gln	Arg	Ala	Tyr	Arg	Arg	Tyr	Leu	Leu	Lys	Gln
1910						1915					1920			
Lys	Val	Lys	Lys	Val	Ser	Ser	Ile	Tyr	Lys	Lys	Asp	Lys	Gly	Lys
1925						1930					1935			
Glu	Cys	Asp	Gly	Thr	Pro	Ile	Lys	Glu	Asp	Thr	Leu	Ile	Asp	Lys
1940						1945					1950			
Leu	Asn	Glu	Asn	Ser	Thr	Pro	Glu	Lys	Thr	Asp	Met	Thr	Pro	Ser
1955						1960					1965			
Thr	Thr	Ser	Pro	Pro	Ser	Tyr	Asp	Ser	Val	Thr	Lys	Pro	Glu	Lys
1970						1975					1980			
Glu	Lys	Phe	Glu	Lys	Asp	Lys	Ser	Glu	Lys	Glu	Asp	Lys	Gly	Lys
1985						1990					1995			
Asp	Ile	Arg	Glu	Ser	Lys	Lys								

2000

## SequencesSSCPre-file7August03.ST25.txt

2005

<210> 67  
<211> 2005  
<212> PRT  
<213> Homo sapiens

&lt;400&gt; 67

Met Ala Gln Ser Val Leu Val Pro Pro Gly Pro Asp Ser Phe Arg Phe  
1 5 10 15  
Phe Thr Arg Glu Ser Leu Ala Ala Ile Glu Gln Arg Ile Ala Glu Glu  
20 25 30  
Lys Ala Lys Arg Pro Lys Gln Glu Arg Lys Asp Glu Asp Asp Glu Asn  
35 40 45  
Gly Pro Lys Pro Asn Ser Asp Leu Glu Ala Gly Lys Ser Leu Pro Phe  
50 55 60  
Ile Tyr Gly Asp Ile Pro Pro Glu Met Val Ser Val Pro Leu Glu Asp  
65 70 75 80  
Leu Asp Pro Tyr Tyr Ile Asn Lys Lys Thr Phe Ile Val Leu Asn Lys  
85 90 95  
Gly Lys Ala Ile Ser Arg Phe Ser Ala Thr Pro Ala Leu Tyr Ile Leu  
100 105 110  
Thr Pro Phe Asn Pro Ile Arg Lys Leu Ala Ile Lys Ile Leu Val His  
115 120 125  
Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val  
130 135 140  
Phe Met Thr Met Ser Asn Pro Pro Asp Trp Thr Lys Asn Val Glu Tyr  
145 150 155 160  
Thr Phe Thr Gly Ile Tyr Thr Phe Glu Ser Leu Ile Lys Ile Leu Ala  
165 170 175  
Arg Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Asp Pro Trp Asn

Trp Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val  
195 200 205

Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg Ala  
210 215 220

Leu Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala  
225 230 235 240

Leu Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val  
245 250 255

Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly  
260 265 270

Asn Leu Arg Asn Lys Cys Leu Gln Trp Pro Pro Asp Asn Ser Ser Phe  
275 280 285

Glu Ile Asn Ile Thr Ser Phe Phe Asn Asn Ser Leu Asp Gly Asn Gly  
290 295 300

Thr Thr Phe Asn Arg Thr Val Ser Ile Phe Asn Trp Asp Glu Tyr Ile  
305 310 315 320

Glu Asp Lys Ser His Phe Tyr Phe Leu Glu Gly Gln Asn Asp Ala Leu  
325 330 335

Leu Cys Gly Asn Ser Ser Asp Ala Gly Gln Cys Pro Glu Gly Tyr Ile  
340 345 350

Cys Val Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp  
355 360 365

Thr Phe Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp  
370 375 380

Phe Trp Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr  
385 390 395 400

SequencesSSCPre-file7August03.ST25.txt

Tyr Met Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu  
405 410 415

Ile Asn Leu Ile Leu Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn  
420 425 430

Gln Ala Thr Leu Glu Glu Ala Glu Gln Lys Glu Ala Glu Phe Gln Gln  
435 440 445

Met Leu Glu Gln Leu Lys Lys Gln Gln Glu Glu Ala Gln Ala Ala Ala  
450 455 460

Ala Ala Ala Ser Ala Glu Ser Arg Asp Phe Ser Gly Ala Gly Gly Ile  
465 470 475 480

Gly Val Phe Ser Glu Ser Ser Ser Val Ala Ser Lys Leu Ser Ser Lys  
485 490 495

Ser Glu Lys Glu Leu Lys Asn Arg Arg Lys Lys Lys Lys Gln Lys Glu  
500 505 510

Gln Ser Gly Glu Glu Glu Lys Asn Asp Arg Val Leu Lys Ser Glu Ser  
515 520 525

Glu Asp Ser Ile Arg Arg Lys Gly Phe Arg Phe Ser Leu Glu Gly Ser  
530 535 540

Arg Leu Thr Tyr Glu Lys Arg Phe Ser Ser Pro His Gln Ser Leu Leu  
545 550 555 560

Ser Ile Arg Gly Ser Leu Phe Ser Pro Arg Arg Asn Ser Arg Ala Ser  
565 570 575

Leu Phe Ser Phe Arg Gly Arg Ala Lys Asp Ile Gly Ser Glu Asn Asp  
580 585 590

Phe Ala Asp Asp Glu His Ser Thr Phe Glu Asp Asn Asp Ser Arg Arg  
595 600 605

Asp Ser Leu Phe Val Pro His Arg His Gly Glu Arg Arg His Ser Asn  
610 615 620

SequencesSSCPre-file7August03.ST25.txt

Val	Ser	Gln	Ala	Ser	Arg	Ala	Ser	Arg	Val	Leu	Pro	Ile	Leu	Pro	Met	
625					630					635					640	
Asn	Gly	Lys	Met	His	Ser	Ala	Val	Asp	Cys	Asn	Gly	Val	Val	Ser	Leu	
				645					650					655		
Val	Gly	Gly	Pro	Ser	Thr	Leu	Thr	Ser	Ala	Gly	Gln	Leu	Leu	Pro	Glu	
			660					665					670			
Gly	Thr	Thr	Thr	Glu	Thr	Glu	Ile	Arg	Lys	Arg	Arg	Ser	Ser	Ser	Tyr	
			675				680					685				
His	Val	Ser	Met	Asp	Leu	Leu	Glu	Asp	Pro	Thr	Ser	Arg	Gln	Arg	Ala	
	690					695					700					
Met	Ser	Ile	Ala	Ser	Ile	Leu	Thr	Asn	Thr	Met	Glu	Glu	Leu	Glu	Glu	
705					710					715					720	
Ser	Arg	Gln	Lys	Cys	Pro	Pro	Cys	Trp	Tyr	Lys	Phe	Ala	Asn	Met	Cys	
				725					730					735		
Leu	Ile	Trp	Asp	Cys	Cys	Lys	Pro	Trp	Leu	Lys	Val	Lys	His	Leu	Val	
			740					745					750			
Asn	Leu	Val	Val	Met	Asp	Pro	Phe	Val	Asp	Leu	Ala	Ile	Thr	Ile	Cys	
		755					760					765				
Ile	Val	Leu	Asn	Thr	Leu	Phe	Met	Ala	Met	Glu	His	Tyr	Pro	Met	Thr	
	770					775					780					
Glu	Gln	Phe	Ser	Ser	Val	Leu	Ser	Val	Gly	Asn	Leu	Val	Phe	Thr	Gly	
785					790				795						800	
Ile	Phe	Thr	Ala	Glu	Met	Phe	Leu	Lys	Ile	Ile	Ala	Met	Asp	Pro	Tyr	
				805					810					815		
Tyr	Tyr	Phe	Gln	Glu	Gly	Trp	Asn	Ile	Phe	Asp	Gly	Phe	Ile	Val	Ser	
			820					825					830			
Leu	Ser	Leu	Met	Glu	Leu	Gly	Leu	Ala	Asn	Val	Glu	Gly	Leu	Ser	Val	
		835					840					845				

SequencesSSCPre-file7August03.ST25.txt

Leu Arg Ser Phe Arg Leu Leu Arg Val Phe Lys Leu Ala Lys Ser Trp  
850 855 860

Pro Thr Leu Asn Met Leu Ile Lys Ile Ile Gly Asn Ser Val Gly Ala  
865 870 875 880

Leu Gly Asn Leu Thr Leu Val Leu Ala Ile Ile Val Phe Ile Phe Ala  
885 890 895

Val Val Gly Met Gln Leu Phe Gly Lys Ser Tyr Lys Glu Cys Val Cys  
900 905 910

Lys Ile Ser Asn Asp Cys Glu Leu Pro Arg Trp His Met His Asp Phe  
915 920 925

Phe His Ser Phe Leu Ile Val Phe Arg Val Leu Cys Gly Glu Trp Ile  
930 935 940

Glu Thr Met Trp Asp Cys Met Glu Val Ala Gly Gln Thr Met Cys Leu  
945 950 955 960

Thr Val Phe Met Met Val Met Val Ile Gly Asn Leu Val Val Leu Asn  
965 970 975

Leu Phe Leu Ala Leu Leu Leu Ser Ser Phe Ser Ser Asp Asn Leu Ala  
980 985 990

Ala Thr Asp Asp Asp Asn Glu Met Asn Asn Leu Gln Ile Ala Val Gly  
995 1000 1005

Arg Met Gln Lys Gly Ile Asp Phe Val Lys Arg Lys Ile Arg Glu  
1010 1015 1020

Phe Ile Gln Lys Ala Phe Val Arg Lys Gln Lys Ala Leu Asp Glu  
1025 1030 1035

Ile Lys Pro Leu Glu Asp Leu Asn Asn Lys Lys Asp Ser Cys Ile  
1040 1045 1050

Ser Asn His Thr Thr Ile Glu Ile Gly Lys Asp Leu Asn Tyr Leu



## SequencesSSCPre-file7August03.ST25.txt

1055

1060

1065

Lys Asp Gly Asn Gly Thr Thr Ser Gly Ile Gly Ser Ser Val Glu  
1070 1075 1080

Lys Tyr Val Val Asp Glu Ser Asp Tyr Met Ser Phe Ile Asn Asn  
1085 1090 1095

Pro Ser Leu Thr Val Thr Val Pro Ile Ala Val Gly Glu Ser Asp  
1100 1105 1110

Phe Glu Asn Leu Asn Thr Glu Glu Phe Ser Ser Glu Ser Asp Met  
1115 1120 1125

Glu Glu Ser Lys Glu Lys Leu Asn Ala Thr Ser Ser Ser Glu Gly  
1130 1135 1140

Ser Thr Val Asp Ile Gly Ala Pro Ala Glu Gly Glu Gln Pro Glu  
1145 1150 1155

Val Glu Pro Glu Glu Ser Leu Glu Pro Glu Ala Cys Phe Thr Glu  
1160 1165 1170

Asp Cys Val Arg Lys Phe Lys Cys Cys Gln Ile Ser Ile Glu Glu  
1175 1180 1185

Gly Lys Gly Lys Leu Trp Trp Asn Leu Arg Lys Ala Cys Tyr Lys  
1190 1195 1200

Ile Val Glu His Asn Trp Phe Glu Thr Phe Ile Val Phe Met Ile  
1205 1210 1215

Leu Leu Ser Ser Gly Ala Leu Ala Phe Glu Asp Ile Tyr Ile Glu  
1220 1225 1230

Gln Arg Lys Thr Ile Lys Thr Met Leu Glu Tyr Ala Asp Lys Val  
1235 1240 1245

Phe Thr Tyr Ile Phe Ile Leu Glu Met Leu Leu Lys Trp Val Ala  
1250 1255 1260

SequencesSSCPre-file7August03.ST25.txt

Tyr Gly	Phe Gln Val Tyr Phe	Thr Asn Ala Trp Cys	Trp Leu Asp
1265		1270	1275
Phe Leu	Ile Val Asp Val Ser	Leu Val Ser Leu Thr	Ala Asn Ala
1280		1285	1290
Leu Gly	Tyr Ser Glu Leu Gly	Ala Ile Lys Ser Leu	Arg Thr Leu
1295		1300	1305
Arg Ala	Leu Arg Pro Leu Arg	Ala Leu Ser Arg Phe	Glu Gly Met
1310		1315	1320
Arg Val	Val Val Asn Ala Leu	Leu Gly Ala Ile Pro	Ser Ile Met
1325		1330	1335
Asn Val	Leu Leu Val Cys Leu	Ile Phe Trp Leu Ile	Phe Ser Ile
1340		1345	1350
Met Gly	Val Asn Leu Phe Ala	Gly Lys Phe Tyr His	Cys Ile Asn
1355		1360	1365
Tyr Thr	Thr Gly Glu Met Phe	Asp Val Ser Val Val	Asn Asn Tyr
1370		1375	1380
Ser Glu	Cys Lys Ala Leu Ile	Glu Ser Asn Gln Thr	Ala Arg Trp
1385		1390	1395
Lys Asn	Val Lys Val Asn Phe	Asp Asn Val Gly Leu	Gly Tyr Leu
1400		1405	1410
Ser Leu	Leu Gln Val Ala Thr	Phe Lys Gly Trp Met	Asp Ile Met
1415		1420	1425
Tyr Ala	Ala Val Asp Ser Arg	Asn Val Glu Leu Gln	Pro Lys Tyr
1430		1435	1440
Glu Asp	Asn Leu Tyr Met Tyr	Leu Tyr Phe Val Ile	Phe Ile Ile
1445		1450	1455
Phe Gly	Ser Phe Phe Thr Leu	Asn Leu Phe Ile Gly	Val Ile Ile
1460		1465	1470

SequencesSSCPre-file7August03.ST25.txt

Asp	Asn	Phe	Asn	Gln	Gln	Lys	Lys	Lys	Phe	Gly	Gly	Gln	Asp	Ile
1475						1480					1485			
Phe	Met	Thr	Glu	Glu	Gln	Lys	Lys	Tyr	Tyr	Asn	Ala	Met	Lys	Lys
1490						1495					1500			
Leu	Gly	Ser	Lys	Lys	Pro	Gln	Lys	Pro	Ile	Pro	Arg	Pro	Ala	Asn
1505						1510					1515			
Lys	Phe	Gln	Gly	Met	Val	Phe	Asp	Phe	Val	Thr	Lys	Gln	Val	Phe
1520						1525					1530			
Asp	Ile	Ser	Ile	Met	Ile	Leu	Ile	Cys	Leu	Asn	Met	Val	Thr	Met
1535						1540					1545			
Met	Val	Glu	Thr	Asp	Asp	Gln	Ser	Gln	Glu	Met	Thr	Asn	Ile	Leu
1550						1555					1560			
Tyr	Trp	Ile	Asn	Leu	Val	Phe	Ile	Val	Leu	Phe	Thr	Gly	Glu	Cys
1565						1570					1575			
Val	Leu	Lys	Leu	Ile	Ser	Leu	Arg	Tyr	Tyr	Tyr	Phe	Thr	Ile	Gly
1580						1585					1590			
Trp	Asn	Ile	Phe	Asp	Phe	Val	Val	Val	Ile	Leu	Ser	Ile	Val	Gly
1595						1600					1605			
Met	Phe	Leu	Ala	Glu	Leu	Ile	Glu	Lys	Tyr	Phe	Val	Ser	Pro	Thr
1610						1615					1620			
Leu	Phe	Arg	Val	Ile	Arg	Leu	Ala	Arg	Ile	Gly	Arg	Ile	Leu	Arg
1625						1630					1635			
Leu	Ile	Lys	Gly	Ala	Lys	Gly	Ile	Arg	Thr	Leu	Leu	Phe	Ala	Leu
1640						1645					1650			
Met	Met	Ser	Leu	Pro	Ala	Leu	Phe	Asn	Ile	Gly	Leu	Leu	Leu	Phe
1655						1660					1665			
Leu	Val	Met	Phe	Ile	Tyr	Ala	Ile	Phe	Gly	Met	Ser	Asn	Phe	Ala
1670						1675					1680			

SequencesSSCPre-file7August03.ST25.txt

Tyr Val Lys Arg Glu Val Gly Ile Asp Asp Met Phe Asn Phe Glu  
1685 1690 1695

Thr Phe Gly Asn Ser Met Ile Cys Leu Phe Gln Ile Thr Thr Ser  
1700 1705 1710

Ala Gly Trp Asp Gly Leu Leu Ala Pro Ile Leu Asn Ser Gly Pro  
1715 1720 1725

Pro Asp Cys Asp Pro Asp Lys Asp His Pro Gly Ser Ser Val Lys  
1730 1735 1740

Gly Asp Cys Gly Asn Pro Ser Val Gly Ile Phe Phe Phe Val Ser  
1745 1750 1755

Tyr Ile Ile Ile Ser Phe Leu Val Val Leu Asn Met Tyr Ile Ala  
1760 1765 1770

Val Ile Leu Glu Asn Phe Ser Val Ala Thr Glu Glu Ser Ala Glu  
1775 1780 1785

Pro Leu Ser Glu Asp Asp Phe Glu Met Phe Tyr Glu Val Trp Glu  
1790 1795 1800

Lys Phe Asp Pro Asp Ala Thr Gln Phe Ile Glu Phe Ala Lys Leu  
1805 1810 1815

Ser Asp Phe Ala Asp Ala Leu Asp Pro Pro Leu Leu Ile Ala Lys  
1820 1825 1830

Pro Asn Lys Val Gln Leu Ile Ala Met Asp Leu Pro Met Val Ser  
1835 1840 1845

Gly Asp Arg Ile His Cys Leu Asp Ile Leu Phe Ala Phe Thr Lys  
1850 1855 1860

Arg Val Leu Gly Glu Ser Gly Glu Met Asp Ala Leu Arg Ile Gln  
1865 1870 1875

Met Glu Glu Arg Phe Met Ala Ser Asn Pro Ser Lys Val Ser Tyr

SequencesSSCPre-file7August03.ST25.txt

1880

1885

1890

Glu Pro Ile Thr Thr Thr Leu Lys Arg Lys Gln Glu Glu Val Ser  
1895 1900 1905

Ala Ile Ile Ile Gln Arg Ala Tyr Arg Arg Tyr Leu Leu Lys Gln  
1910 1915 1920

Lys Val Lys Lys Val Ser Ser Ile Tyr Lys Lys Asp Lys Gly Lys  
1925 1930 1935

Glu Cys Asp Gly Thr Pro Ile Lys Glu Asp Thr Leu Ile Asp Lys  
1940 1945 1950

Leu Asn Glu Asn Ser Thr Pro Glu Lys Thr Asp Met Thr Pro Ser  
1955 1960 1965

Thr Thr Ser Pro Pro Ser Tyr Asp Ser Val Thr Lys Pro Glu Lys  
1970 1975 1980

Glu Lys Phe Glu Lys Asp Lys Ser Glu Lys Glu Asp Lys Gly Lys  
1985 1990 1995

Asp Ile Arg Glu Ser Lys Lys  
2000 2005

<210> 68  
<211> 2005  
<212> PRT  
<213> Homo sapiens

<400> 68

Met Ala Gln Ser Val Leu Val Pro Pro Gly Pro Asp Ser Phe Arg Phe  
1 5 10 15

Phe Thr Arg Glu Ser Leu Ala Ala Ile Glu Gln Arg Ile Ala Glu Glu  
20 25 30

Lys Ala Lys Arg Pro Lys Gln Glu Arg Lys Asp Glu Asp Asp Glu Asn  
35 40 45

Gly Pro Lys Pro Asn Ser Asp Leu Glu Ala Gly Lys Ser Leu Pro Phe

## SequencesSSCPre-file7August03.ST25.txt

50

55

60

Ile Tyr Gly Asp Ile Pro Pro Glu Met Val Ser Val Pro Leu Glu Asp  
65 70 75 80

Leu Asp Pro Tyr Tyr Ile Asn Lys Lys Thr Phe Ile Val Leu Asn Lys  
85 90 95

Gly Lys Ala Ile Ser Arg Phe Ser Ala Thr Pro Ala Leu Tyr Ile Leu  
100 105 110

Thr Pro Phe Asn Pro Ile Arg Lys Leu Ala Ile Lys Ile Leu Val His  
115 120 125

Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val  
130 135 140

Phe Met Thr Met Ser Asn Pro Pro Asp Trp Thr Lys Asn Val Glu Tyr  
145 150 155 160

Thr Phe Thr Gly Ile Tyr Thr Phe Glu Ser Leu Ile Lys Ile Leu Ala  
165 170 175

Arg Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Asp Pro Trp Asn  
180 185 190

Trp Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val  
195 200 205

Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg Ala  
210 215 220

Leu Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala  
225 230 235 240

Leu Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val  
245 250 255

Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly  
260 265 270

SequencesSSCPre-file7August03.ST25.txt

Asn Leu Arg Asn Lys Cys Leu Gln Trp Pro Pro Asp Asn Ser Ser Phe  
275 280 285

Glu Ile Asn Ile Thr Ser Phe Phe Asn Asn Ser Leu Asp Gly Asn Gly  
290 295 300

Thr Thr Phe Asn Arg Thr Val Ser Ile Phe Asn Trp Asp Glu Tyr Ile  
305 310 315 320

Glu Asp Lys Ser His Phe Tyr Phe Leu Glu Gly Gln Asn Asp Ala Leu  
325 330 335

Leu Cys Gly Asn Ser Ser Asp Ala Gly Gln Cys Pro Glu Gly Tyr Ile  
340 345 350

Cys Val Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp  
355 360 365

Thr Phe Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp  
370 375 380

Phe Trp Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr  
385 390 395 400

Tyr Met Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu  
405 410 415

Ile Asn Leu Ile Leu Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn  
420 425 430

Gln Ala Thr Leu Glu Glu Ala Glu Gln Lys Glu Ala Glu Phe Gln Gln  
435 440 445

Met Leu Glu Gln Leu Lys Lys Gln Gln Glu Glu Ala Gln Ala Ala Ala  
450 455 460

Ala Ala Ala Ser Ala Glu Ser Arg Asp Phe Ser Gly Ala Gly Gly Ile  
465 470 475 480

Gly Val Phe Ser Glu Ser Ser Ser Val Ala Ser Lys Leu Ser Ser Lys  
485 490 495

SequencesSSCPre-file7August03.ST25.txt

Ser Glu Lys Glu Leu Lys Asn Arg Arg Lys Lys Lys Lys Gln Lys Glu  
500 505 510

Gln Ser Gly Glu Glu Glu Lys Asn Asp Arg Val Leu Lys Ser Glu Ser  
515 520 525

Glu Asp Ser Ile Arg Arg Lys Gly Phe Arg Phe Ser Leu Glu Gly Ser  
530 535 540

Arg Leu Thr Tyr Glu Lys Arg Phe Ser Ser Pro His Gln Ser Leu Leu  
545 550 555 560

Ser Ile Arg Gly Ser Leu Phe Ser Pro Arg Arg Asn Ser Arg Ala Ser  
565 570 575

Leu Phe Ser Phe Arg Gly Arg Ala Lys Asp Ile Gly Ser Glu Asn Asp  
580 585 590

Phe Ala Asp Asp Glu His Ser Thr Phe Glu Asp Asn Asp Ser Arg Arg  
595 600 605

Asp Ser Leu Phe Val Pro His Arg His Gly Glu Arg Arg His Ser Asn  
610 615 620

Val Ser Gln Ala Ser Arg Ala Ser Arg Val Leu Pro Ile Leu Pro Met  
625 630 635 640

Asn Gly Lys Met His Ser Ala Val Asp Cys Asn Gly Val Val Ser Leu  
645 650 655

Val Gly Gly Pro Ser Thr Leu Thr Ser Ala Gly Gln Leu Leu Pro Glu  
660 665 670

Gly Thr Thr Thr Glu Thr Glu Ile Arg Lys Arg Arg Ser Ser Ser Tyr  
675 680 685

His Val Ser Met Asp Leu Leu Glu Asp Pro Thr Ser Arg Gln Arg Ala  
690 695 700

Met Ser Ile Ala Ser Ile Leu Thr Asn Thr Met Glu Glu Leu Glu Glu  
705 710 715 720



SequencesSSCPre-file7August03.ST25.txt

Ser Arg Gln Lys Cys Pro Pro Cys Trp Tyr Lys Phe Ala Asn Met Cys  
725 730 735

Leu Ile Trp Asp Cys Cys Lys Pro Trp Leu Lys Val Lys His Leu Val  
740 745 750

Asn Leu Val Val Met Asp Pro Phe Val Asp Leu Ala Ile Thr Ile Cys  
755 760 765

Ile Val Leu Asn Thr Leu Phe Met Ala Met Glu His Tyr Pro Met Thr  
770 775 780

Glu Gln Phe Ser Ser Val Leu Ser Val Gly Asn Leu Val Phe Thr Gly  
785 790 795 800

Ile Phe Thr Ala Glu Met Phe Leu Lys Ile Ile Ala Met Asp Pro Tyr  
805 810 815

Tyr Tyr Phe Gln Glu Gly Trp Asn Ile Phe Asp Gly Phe Ile Val Ser  
820 825 830

Leu Ser Leu Met Glu Leu Gly Leu Ala Asn Val Glu Gly Leu Ser Val  
835 840 845

Leu Arg Ser Phe Arg Leu Leu Arg Val Phe Lys Leu Ala Lys Ser Trp  
850 855 860

Pro Thr Leu Asn Met Leu Ile Lys Ile Ile Gly Asn Ser Val Gly Ala  
865 870 875 880

Leu Gly Asn Leu Thr Leu Val Leu Ala Ile Ile Val Phe Ile Phe Ala  
885 890 895

Val Val Gly Met Gln Leu Phe Gly Lys Ser Tyr Lys Glu Cys Val Cys  
900 905 910

Lys Ile Ser Asn Asp Cys Glu Leu Pro Arg Trp His Met His Asp Phe  
915 920 925

Phe His Ser Phe Leu Ile Val Phe Arg Val Leu Cys Gly Glu Trp Ile

SequencesSSCPre-file7August03.ST25.txt

930

935

940

Glu Thr Met Trp Asp Cys Met Glu Val Ala Gly Gln Thr Met Cys Leu  
945 950 955 960

Thr Val Phe Met Met Val Met Val Ile Gly Asn Leu Val Val Leu Asn  
965 970 975

Leu Phe Leu Ala Leu Leu Leu Ser Ser Phe Ser Ser Asp Asn Leu Ala  
980 985 990

Ala Thr Asp Asp Asp Asn Glu Met Asn Asn Leu Gln Ile Ala Val Gly  
995 1000 1005

Arg Met Gln Lys Gly Ile Asp Phe Val Lys Arg Lys Ile Arg Glu  
1010 1015 1020

Phe Ile Gln Lys Ala Phe Val Arg Lys Gln Lys Ala Leu Asp Glu  
1025 1030 1035

Ile Lys Pro Leu Glu Asp Leu Asn Asn Lys Lys Asp Ser Cys Ile  
1040 1045 1050

Ser Asn His Thr Thr Ile Glu Ile Gly Lys Asp Leu Asn Tyr Leu  
1055 1060 1065

Lys Asp Gly Asn Gly Thr Thr Ser Gly Ile Gly Ser Ser Val Glu  
1070 1075 1080

Lys Tyr Val Val Asp Glu Ser Asp Tyr Met Ser Phe Ile Asn Asn  
1085 1090 1095

Pro Ser Leu Thr Val Thr Val Pro Ile Ala Val Gly Glu Ser Asp  
1100 1105 1110

Phe Glu Asn Leu Asn Thr Glu Glu Phe Ser Ser Glu Ser Asp Met  
1115 1120 1125

Glu Glu Ser Lys Glu Lys Leu Asn Ala Thr Ser Ser Ser Glu Gly  
1130 1135 1140

SequencesSSCPre-file7August03.ST25.txt

Ser Thr Val Asp Ile Gly Ala Pro Ala Glu Gly Glu Gln Pro Glu  
1145 1150 1155

Val Glu Pro Glu Glu Ser Leu Glu Pro Glu Ala Cys Phe Thr Glu  
1160 1165 1170

Asp Cys Val Arg Lys Phe Lys Cys Cys Gln Ile Ser Ile Glu Glu  
1175 1180 1185

Gly Lys Gly Lys Leu Trp Trp Asn Leu Arg Lys Thr Cys Tyr Lys  
1190 1195 1200

Ile Val Glu His Asn Trp Phe Glu Thr Phe Ile Val Phe Met Ile  
1205 1210 1215

Leu Leu Ser Ser Gly Ala Leu Ala Phe Glu Asp Ile Tyr Ile Glu  
1220 1225 1230

Gln Arg Lys Thr Ile Lys Thr Met Leu Glu Tyr Ala Asp Lys Val  
1235 1240 1245

Phe Thr Tyr Ile Phe Ile Leu Glu Met Leu Leu Lys Trp Val Ala  
1250 1255 1260

Tyr Gly Phe Gln Val Tyr Phe Thr Asn Ala Trp Cys Trp Leu Asp  
1265 1270 1275

Phe Leu Ile Val Asp Val Ser Leu Val Ser Leu Thr Ala Asn Ala  
1280 1285 1290

Leu Gly Tyr Ser Glu Leu Gly Ala Ile Lys Ser Leu Arg Thr Leu  
1295 1300 1305

Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser Gln Phe Glu Gly Met  
1310 1315 1320

Arg Val Val Val Asn Ala Leu Leu Gly Ala Ile Pro Ser Ile Met  
1325 1330 1335

Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Ile Phe Ser Ile  
1340 1345 1350

SequencesSSCPre-file7August03.ST25.txt

Met Gly Val Asn Leu Phe Ala Gly Lys Phe Tyr His Cys Ile Asn  
1355 1360 1365

Tyr Thr Thr Gly Glu Met Phe Asp Val Ser Val Val Asn Asn Tyr  
1370 1375 1380

Ser Glu Cys Lys Ala Leu Ile Glu Ser Asn Gln Thr Ala Arg Trp  
1385 1390 1395

Lys Asn Val Lys Val Asn Phe Asp Asn Val Gly Leu Gly Tyr Leu  
1400 1405 1410

Ser Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp Ile Met  
1415 1420 1425

Tyr Ala Ala Val Asp Ser Arg Asn Val Glu Leu Gln Pro Lys Tyr  
1430 1435 1440

Glu Asp Asn Leu Tyr Met Tyr Leu Tyr Phe Val Ile Phe Ile Ile  
1445 1450 1455

Phe Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile Gly Val Ile Ile  
1460 1465 1470

Asp Asn Phe Asn Gln Gln Lys Lys Lys Phe Gly Gly Gln Asp Ile  
1475 1480 1485

Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys  
1490 1495 1500

Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro Ala Asn  
1505 1510 1515

Lys Phe Gln Gly Met Val Phe Asp Phe Val Thr Lys Gln Val Phe  
1520 1525 1530

Asp Ile Ser Ile Met Ile Leu Ile Cys Leu Asn Met Val Thr Met  
1535 1540 1545

Met Val Glu Thr Asp Asp Gln Ser Gln Glu Met Thr Asn Ile Leu  
1550 1555 1560

SequencesSSCPre-file7August03.ST25.txt

Tyr Trp Ile Asn Leu Val Phe Ile Val Leu Phe Thr Gly Glu Cys  
 1565 1570 1575  
 Val Leu Lys Leu Ile Ser Leu Arg Tyr Tyr Tyr Phe Thr Ile Gly  
 1580 1585 1590  
 Trp Asn Ile Phe Asp Phe Val Val Val Ile Leu Ser Ile Val Gly  
 1595 1600 1605  
 Met Phe Leu Ala Glu Leu Ile Glu Lys Tyr Phe Val Ser Pro Thr  
 1610 1615 1620  
 Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg  
 1625 1630 1635  
 Leu Ile Lys Gly Ala Lys Gly Ile Arg Thr Leu Leu Phe Ala Leu  
 1640 1645 1650  
 Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu Phe  
 1655 1660 1665  
 Leu Val Met Phe Ile Tyr Ala Ile Phe Gly Met Ser Asn Phe Ala  
 1670 1675 1680  
 Tyr Val Lys Arg Glu Val Gly Ile Asp Asp Met Phe Asn Phe Glu  
 1685 1690 1695  
 Thr Phe Gly Asn Ser Met Ile Cys Leu Phe Gln Ile Thr Thr Ser  
 1700 1705 1710  
 Ala Gly Trp Asp Gly Leu Leu Ala Pro Ile Leu Asn Ser Gly Pro  
 1715 1720 1725  
 Pro Asp Cys Asp Pro Asp Lys Asp His Pro Gly Ser Ser Val Lys  
 1730 1735 1740  
 Gly Asp Cys Gly Asn Pro Ser Val Gly Ile Phe Phe Phe Val Ser  
 1745 1750 1755  
 Tyr Ile Ile Ile Ser Phe Leu Val Val Leu Asn Met Tyr Ile Ala  
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## SequencesSSCPre-file7August03.ST25.txt

1760

1765

1770

Val Ile Leu Glu Asn Phe Ser Val Ala Thr Glu Glu Ser Ala Glu  
 1775 1780 1785

Pro Leu Ser Glu Asp Asp Phe Glu Met Phe Tyr Glu Val Trp Glu  
 1790 1795 1800

Lys Phe Asp Pro Asp Ala Thr Gln Phe Ile Glu Phe Ala Lys Leu  
 1805 1810 1815

Ser Asp Phe Ala Asp Ala Leu Asp Pro Pro Leu Leu Ile Ala Lys  
 1820 1825 1830

Pro Asn Lys Val Gln Leu Ile Ala Met Asp Leu Pro Met Val Ser  
 1835 1840 1845

Gly Asp Arg Ile His Cys Leu Asp Ile Leu Phe Ala Phe Thr Lys  
 1850 1855 1860

Arg Val Leu Gly Glu Ser Gly Glu Met Asp Ala Leu Arg Ile Gln  
 1865 1870 1875

Met Glu Glu Arg Phe Met Ala Ser Asn Pro Ser Lys Val Ser Tyr  
 1880 1885 1890

Glu Pro Ile Thr Thr Thr Leu Lys Arg Lys Gln Glu Glu Val Ser  
 1895 1900 1905

Ala Ile Ile Ile Gln Arg Ala Tyr Arg Arg Tyr Leu Leu Lys Gln  
 1910 1915 1920

Lys Val Lys Lys Val Ser Ser Ile Tyr Lys Lys Asp Lys Gly Lys  
 1925 1930 1935

Glu Cys Asp Gly Thr Pro Ile Lys Glu Asp Thr Leu Ile Asp Lys  
 1940 1945 1950

Leu Asn Glu Asn Ser Thr Pro Glu Lys Thr Asp Met Thr Pro Ser  
 1955 1960 1965

SequencesSSCPre-file7August03.ST25.txt

Thr Thr Ser Pro Pro Ser Tyr Asp Ser Val Thr Lys Pro Glu Lys  
1970 1975 1980

Glu Lys Phe Glu Lys Asp Lys Ser Glu Lys Glu Asp Lys Gly Lys  
1985 1990 1995

Asp Ile Arg Glu Ser Lys Lys  
2000 2005

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<212> PRT  
<213> Homo sapiens

<400> 69

Met Ala Ala Arg Gly Ser Gly Pro Arg Ala Leu Arg Leu Leu Leu Leu  
1 5 10 15

Val Gln Leu Val Ala Gly Ala Leu Arg Ser Ser Arg Ala Arg Arg Ala  
20 25 30

Ala Arg Arg Gly Leu Ser Glu Pro Ser Ser Ile Ala Lys His Glu Asp  
35 40 45

Ser Leu Leu Lys Asp Leu Phe Gln Asp Tyr Glu Arg Trp Val Arg Pro  
50 55 60

Val Glu His Leu Asn Asp Lys Ile Lys Ile Lys Phe Gly Leu Ala Ile  
65 70 75 80

Ser Gln Leu Val Asp Val Asp Glu Lys Asn Gln Leu Met Thr Thr Asn  
85 90 95

Val Trp Leu Lys Gln Glu Trp Ile Asp Val Lys Leu Arg Trp Asn Pro  
100 105 110

Asp Asp Tyr Gly Gly Ile Lys Val Ile Arg Val Pro Ser Asp Ser Ser  
115 120 125

Trp Thr Pro Asp Ile Ile Leu Phe Asp Asn Ala Asp Gly Arg Phe Glu  
130 135 140

SequencesSSCPre-file7August03.ST25.txt

Gly Thr Ser Thr Lys Thr Val Ile Arg Tyr Asn Gly Thr Val Thr Trp  
145 150 155 160

Thr Pro Pro Ala Asn Tyr Lys Ser Ser Cys Thr Ile Asp Val Thr Phe  
165 170 175

Phe Pro Phe Asp Leu Gln Asn Cys Ser Met Lys Phe Gly Ser Trp Thr  
180 185 190

Tyr Asp Gly Ser Gln Val Asp Ile Ile Leu Glu Asp Gln Asp Val Asp  
195 200 205

Lys Arg Asp Phe Phe Asp Asn Gly Glu Trp Glu Ile Val Ser Ala Thr  
210 215 220

Gly Ser Lys Gly Asn Arg Thr Asp Ser Cys Cys Trp Tyr Pro Tyr Val  
225 230 235 240

Thr Tyr Ser Phe Val Ile Lys Arg Leu Pro Leu Phe Tyr Thr Leu Phe  
245 250 255

Leu Ile Ile Pro Cys Ile Gly Leu Ser Phe Leu Thr Val Leu Val Phe  
260 265 270

Tyr Leu Pro Ser Asn Glu Gly Glu Lys Ile Cys Leu Cys Thr Ser Val  
275 280 285

Leu Val Ser Leu Thr Val Phe Leu Leu Val Ile Glu Glu Ile Ile Pro  
290 295 300

Ser Ser Ser Lys Val Ile Pro Leu Ile Gly Glu Tyr Leu Val Phe Thr  
305 310 315 320

Met Ile Phe Val Thr Leu Ser Ile Met Val Thr Val Phe Ala Ile Asn  
325 330 335

Ile His His Arg Ser Ser Ser Thr His Asn Ala Met Ala Pro Leu Val  
340 345 350

Arg Lys Ile Phe Leu His Thr Leu Pro Lys Leu Leu Ser Met Arg Ser  
355 360 365



SequencesSSCPre-file7August03.ST25.txt

His Val Asp Arg Tyr Phe Thr Gln Lys Glu Glu Thr Glu Ser Gly Ser  
370 375 380

Gly Pro Lys Ser Ser Arg Asn Thr Leu Glu Ala Ala Leu Asp Ser Ile  
385 390 395 400

Arg Tyr Ile Thr Thr His Ile Met Lys Glu Asn Asp Val Arg Glu Val  
405 410 415

Val Glu Asp Trp Lys Phe Ile Ala Gln Val Leu Asp Arg Met Phe Leu  
420 425 430

Trp Thr Phe Leu Phe Val Ser Ile Val Gly Ser Leu Gly Leu Phe Val  
435 440 445

Pro Val Ile Tyr Lys Trp Ala Asn Ile Leu Ile Pro Val His Ile Gly  
450 455 460

Asn Ala Asn Lys  
465

<210> 70  
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<212> PRT  
<213> Homo sapiens

<400> 70

Met Gly Pro Ser Cys Pro Val Phe Leu Ser Phe Thr Lys Leu Ser Leu  
1 5 10 15

Trp Trp Leu Leu Leu Thr Pro Ala Gly Gly Glu Glu Ala Lys Arg Pro  
20 25 30

Pro Pro Arg Ala Pro Gly Asp Pro Leu Ser Ser Pro Ser Pro Thr Ala  
35 40 45

Leu Pro Gln Gly Gly Ser His Thr Glu Thr Glu Asp Arg Leu Phe Lys  
50 55 60

His Leu Phe Arg Gly Tyr Asn Arg Trp Ala Arg Pro Val Pro Asn Thr  
65 70 75 80

SequencesSSCPre-file7August03.ST25.txt

Ser Asp Val Val Ile Val Arg Phe Gly Leu Ser Ile Ala Gln Leu Ile  
85 90 95

Asp Val Asp Glu Lys Asn Gln Met Met Thr Thr Asn Val Trp Leu Lys  
100 105 110

Gln Glu Trp Ser Asp Tyr Lys Leu Arg Trp Asn Pro Thr Asp Phe Gly  
115 120 125

Asn Ile Thr Ser Leu Arg Val Pro Ser Glu Met Ile Trp Ile Pro Asp  
130 135 140

Ile Val Leu Tyr Asn Asn Ala Asp Gly Glu Phe Ala Val Thr His Met  
145 150 155 160

Thr Lys Ala His Leu Phe Ser Thr Gly Thr Val His Trp Val Pro Pro  
165 170 175

Ala Ile Tyr Lys Ser Ser Cys Ser Ile Asp Val Thr Phe Phe Pro Phe  
180 185 190

Asp Gln Gln Asn Cys Lys Met Lys Phe Gly Ser Trp Thr Tyr Asp Lys  
195 200 205

Ala Lys Ile Asp Leu Glu Gln Met Glu Gln Thr Val Asp Leu Lys Asp  
210 215 220

Tyr Trp Glu Ser Gly Glu Trp Ala Ile Val Asn Ala Thr Gly Thr Tyr  
225 230 235 240

Asn Ser Lys Lys Tyr Asp Cys Cys Ala Glu Ile Tyr Pro Asp Val Thr  
245 250 255

Tyr Ala Phe Val Ile Arg Arg Leu Pro Leu Phe Tyr Thr Ile Asn Leu  
260 265 270

Ile Ile Pro Cys Leu Leu Ile Ser Cys Leu Thr Val Leu Val Phe Tyr  
275 280 285

Leu Pro Ser Asp Cys Gly Glu Lys Ile Thr Leu Cys Ile Ser Val Leu  
290 295 300

SequencesSSCPre-file7August03.ST25.txt

Leu Ser Leu Thr Val Phe Leu Leu Leu Ile Thr Glu Ile Ile Pro Ser  
305 310 315 320

Thr Ser Leu Val Ile Pro Leu Ile Gly Glu Tyr Leu Leu Phe Thr Met  
325 330 335

Ile Phe Val Thr Leu Ser Ile Val Ile Thr Val Phe Val Leu Asn Val  
340 345 350

His His Arg Ser Pro Ser Thr His Thr Met Pro His Trp Val Arg Gly  
355 360 365

Ala Leu Leu Gly Cys Val Pro Arg Trp Leu Leu Met Asn Arg Pro Pro  
370 375 380

Pro Pro Val Glu Leu Cys His Pro Leu Arg Leu Lys Leu Ser Pro Ser  
385 390 395 400

Tyr His Trp Leu Glu Ser Asn Val Asp Ala Glu Glu Arg Glu Val Val  
405 410 415

Val Glu Glu Glu Asp Arg Trp Ala Cys Ala Gly His Val Ala Pro Ser  
420 425 430

Val Gly Thr Leu Cys Ser His Gly His Leu His Ser Gly Ala Ser Gly  
435 440 445

Pro Lys Ala Glu Ala Leu Leu Gln Glu Gly Glu Leu Leu Leu Ser Pro  
450 455 460

His Met Gln Lys Ala Leu Glu Gly Val His Tyr Ile Ala Asp His Leu  
465 470 475 480

Arg Ser Glu Asp Ala Asp Ser Ser Val Lys Glu Asp Trp Lys Tyr Val  
485 490 495

Ala Met Val Ile Asp Arg Ile Phe Leu Trp Leu Phe Ile Ile Val Cys  
500 505 510

Phe Leu Gly Thr Ile Gly Leu Phe Leu Pro Pro Phe Leu Ala Gly Met

Ile

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<212> PRT  
<213> Homo sapiens

<400> 71

Met Gly Ser Gly Pro Leu Ser Leu Pro Leu Ala Leu Ser Pro Pro Arg  
1 5 10 15

Leu Leu Leu Leu Leu Leu Leu Ser Leu Leu Pro Val Ala Arg Ala Ser  
20 25 30

Glu Ala Glu His His Leu Phe Glu Arg Leu Phe Glu Asp Tyr Asn Glu  
35 40 45

Ile Ile Arg Pro Val Ala Asn Val Ser Asp Pro Val Ile Ile His Phe  
50 55 60

Glu Val Ser Met Ser Gln Leu Val Lys Val Asp Glu Val Asn Gln Ile  
65 70 75 80

Met Glu Thr Asn Leu Trp Leu Lys Gln Ile Trp Asn Asp Tyr Lys Leu  
85 90 95

Lys Trp Asn Pro Ser Asp Tyr Gly Gly Ala Glu Phe Met Arg Val Pro  
100 105 110

Ala Gln Lys Ile Trp Lys Pro Asp Ile Val Leu Tyr Asn Asn Ala Val  
115 120 125

Gly Asp Phe Gln Val Asp Asp Lys Thr Lys Ala Leu Leu Lys Tyr Thr  
130 135 140

Gly Glu Val Thr Trp Ile Pro Pro Ala Ile Phe Lys Ser Ser Cys Lys  
145 150 155 160

Ile Asp Val Thr Tyr Phe Pro Phe Asp Tyr Gln Asn Cys Thr Met Lys

SequencesSSCPre-file7August03.ST25.txt

165

170

175

Phe Gly Ser Trp Ser Tyr Asp Lys Ala Lys Ile Asp Leu Val Leu Ile  
180 185 190

Gly Ser Ser Met Asn Leu Lys Asp Tyr Trp Glu Ser Gly Glu Trp Ala  
195 200 205

Ile Ile Lys Ala Pro Gly Tyr Lys His Asp Ile Lys Tyr Asn Cys Cys  
210 215 220

Glu Glu Ile Tyr Pro Asp Ile Thr Tyr Ser Leu Tyr Ile Arg Arg Leu  
225 230 235 240

Pro Leu Phe Tyr Thr Ile Asn Leu Ile Ile Pro Cys Leu Leu Ile Ser  
245 250 255

Phe Leu Thr Val Leu Val Phe Tyr Leu Pro Ser Asp Cys Gly Glu Lys  
260 265 270

Val Thr Leu Cys Ile Ser Val Leu Leu Ser Leu Thr Val Phe Leu Leu  
275 280 285

Val Ile Thr Glu Thr Ile Pro Ser Thr Ser Leu Val Ile Pro Leu Ile  
290 295 300

Gly Glu Tyr Leu Leu Phe Thr Met Ile Phe Val Thr Leu Ser Ile Val  
305 310 315 320

Ile Thr Val Phe Val Leu Asn Val His Tyr Arg Thr Pro Thr Thr His  
325 330 335

Thr Met Pro Ser Trp Val Lys Thr Val Phe Leu Asn Leu Leu Pro Arg  
340 345 350

Val Met Phe Met Thr Arg Pro Thr Ser Asn Glu Gly Asn Ala Gln Lys  
355 360 365

Pro Arg Pro Leu Tyr Gly Ala Glu Leu Ser Asn Leu Asn Cys Phe Ser  
370 375 380

SequencesSSCPre-file7August03.ST25.txt

Arg Ala Glu Ser Lys Gly Cys Lys Glu Gly Tyr Pro Cys Gln Asp Gly  
385 390 395 400

Met Cys Gly Tyr Cys His His Arg Arg Ile Lys Ile Ser Asn Phe Ser  
405 410 415

Ala Asn Leu Thr Arg Ser Ser Ser Ser Glu Ser Val Asp Ala Val Leu  
420 425 430

Ser Leu Ser Ala Leu Ser Pro Glu Ile Lys Glu Ala Ile Gln Ser Val  
435 440 445

Lys Tyr Ile Ala Glu Asn Met Lys Ala Gln Asn Glu Ala Lys Glu Ile  
450 455 460

Gln Asp Asp Trp Lys Tyr Val Ala Met Val Ile Asp Arg Ile Phe Leu  
465 470 475 480

Trp Val Phe Thr Leu Val Cys Ile Leu Gly Thr Ala Gly Leu Phe Leu  
485 490 495

Gln Pro Leu Met Ala Arg Glu Asp Ala  
500 505

<210> 72  
<211> 118  
<212> PRT  
<213> Homo sapiens

<400> 72

Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Pro Ser Gly  
1 5 10 15

Glu Lys Lys Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Ala Pro  
20 25 30

Asp Ser Thr Arg Asp Gly Ala Leu Ile Ala Gly Ser Glu Ala Pro  
35 40 45

Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala Gly Ala  
50 55 60

SequencesSSCPre-file7August03.ST25.txt

Gly Lys Pro Pro Gln Ala Gln Arg Leu Leu Pro Gln Ala Ala Glu Phe  
65 70 75 80

Pro Leu Gln Arg Ala Gly Ala Ala Ala Arg Leu Gly Val His Leu Pro  
85 90 95

Arg Leu Arg Val Pro Pro Gly Phe Leu Leu Pro Arg Ala Val Cys Val  
100 105 110

Phe His His Gln Gly Val  
115

<210> 73  
<211> 854  
<212> PRT  
<213> Homo sapiens  
  
<400> 73

Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Pro Ser Gly  
1 5 10 15

Glu Lys Lys Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Ala Pro  
20 25 30

Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu Ala Pro  
35 40 45

Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala Gly Ala  
50 55 60

Gly Lys Pro Pro Lys Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe  
65 70 75 80

Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile Tyr His  
85 90 95

Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
100 105 110

Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile  
115 120 125

SequencesSSCPre-file7August03.ST25.txt

Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg  
130 135 140

Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg  
145 150 155 160

Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu  
165 170 175

Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe  
180 185 190

Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met  
195 200 205

Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val  
210 215 220

Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile Gly Phe  
225 230 235 240

Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu Lys Gly  
245 250 255

Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu  
260 265 270

Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln Thr Trp  
275 280 285

Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val Ser Phe  
290 295 300

Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val  
305 310 315 320

Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala  
325 330 335

Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn Leu Ser  
340 345 350



SequencesSSCPre-file7August03.ST25.txt

Gly Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Arg Thr Val Thr  
355 360 365

Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser Arg Leu  
370 375 380

Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys Ser Lys  
385 390 395 400

Ser Gly Leu Ala Phe Arg Lys Asp Pro Pro Pro Glu Pro Ser Pro Ser  
405 410 415

Gln Lys Val Ser Leu Lys Asp Arg Val Phe Ser Ser Pro Arg Gly Val  
420 425 430

Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr Val Arg Arg Ser  
435 440 445

Pro Ser Ala Asp Gln Ser Leu Glu Asp Ser Pro Ser Lys Val Pro Lys  
450 455 460

Ser Trp Ser Phe Gly Asp Arg Ser Arg Ala Arg Gln Ala Phe Arg Ile  
465 470 475 480

Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala Ser Leu Pro Gly  
485 490 495

Glu Asp Ile Val Asp Asp Lys Ser Cys Pro Cys Glu Phe Val Thr Glu  
500 505 510

Asp Leu Thr Pro Gly Leu Lys Val Ser Ile Arg Ala Val Cys Val Met  
515 520 525

Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu Ser Leu Arg Pro Tyr  
530 535 540

Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu Asp Met  
545 550 555 560

Leu Ser Arg Ile Lys Ser Leu Gln Ser Arg Val Asp Gln Ile Val Gly  
565 570 575

SequencesSSCPre-file7August03.ST25.txt

Arg Gly Pro Ala Ile Thr Asp Lys Asp Arg Thr Lys Gly Pro Ala Glu  
580 585 590

Ala Glu Leu Pro Glu Asp Pro Ser Met Met Gly Arg Leu Gly Lys Val  
595 600 605

Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu Asp Phe Leu Val Asn  
610 615 620

Ile Tyr Met Gln Arg Met Gly Ile Pro Pro Thr Glu Thr Glu Ala Tyr  
625 630 635 640

Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro Tyr His Ser Pro Glu  
645 650 655

Asp Ser Arg Glu His Val Asp Arg His Gly Cys Ile Val Lys Ile Val  
660 665 670

Arg Ser Ser Ser Ser Thr Gly Gln Lys Asn Phe Ser Ala Pro Pro Ala  
675 680 685

Ala Pro Pro Val Gln Cys Pro Pro Ser Thr Ser Trp Gln Pro Gln Ser  
690 695 700

His Pro Arg Gln Gly His Gly Thr Ser Pro Val Gly Asp His Gly Ser  
705 710 715 720

Leu Val Arg Ile Pro Pro Pro Pro Ala His Glu Arg Ser Leu Ser Ala  
725 730 735

Tyr Gly Gly Gly Asn Arg Ala Ser Met Glu Phe Leu Arg Gln Glu Asp  
740 745 750

Thr Pro Gly Cys Arg Pro Pro Glu Gly Thr Leu Arg Asp Ser Asp Thr  
755 760 765

Ser Ile Ser Ile Pro Ser Val Asp His Glu Glu Leu Glu Arg Ser Phe  
770 775 780

Ser Gly Phe Ser Ile Ser Gln Ser Lys Glu Asn Leu Asp Ala Leu Asn

SequencesSSCPre-file7August03.ST25.txt

785

790

795

800

Ser Cys Tyr Ala Ala Val Ala Pro Cys Ala Lys Val Arg Pro Tyr Ile  
805 810 815

Ala Glu Gly Glu Ser Asp Thr Asp Ser Asp Leu Cys Thr Pro Cys Gly  
820 825 830

Pro Pro Pro Arg Ser Ala Thr Gly Glu Gly Pro Phe Gly Asp Val Gly  
835 840 845

Trp Ala Gly Pro Arg Lys  
850

<210> 74  
<211> 429  
<212> PRT  
<213> Homo sapiens

<400> 74

Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Pro Ser Gly  
1 5 10 15

Glu Lys Lys Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Ala Pro  
20 25 30

Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu Ala Pro  
35 40 45

Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala Gly Ala  
50 55 60

Gly Lys Pro Pro Lys Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe  
65 70 75 80

Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile Tyr His  
85 90 95

Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
100 105 110

Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile

## SequencesSSCPre-file7August03.ST25.txt

115

120

125

Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg  
 130 135 140

Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg  
 145 150 155 160

Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu  
 165 170 175

Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe  
 180 185 190

Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met  
 195 200 205

Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val  
 210 215 220

Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile Gly Phe  
 225 230 235 240

Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu Lys Gly  
 245 250 255

Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu  
 260 265 270

Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln Thr Trp  
 275 280 285

Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val Ser Phe  
 290 295 300

Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val  
 305 310 315 320

Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala  
 325 330 335

SequencesSSCPre-file7August03.ST25.txt

Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn Leu Ser  
340 345 350

Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Arg Thr Val Thr  
355 360 365

Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser Arg Leu  
370 375 380

Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys Ser Lys  
385 390 395 400

Ser Gly Leu Ala Phe Arg Lys Asp Pro Pro Pro Glu Pro Ser Pro Ser  
405 410 415

Gln Lys Val Ser Leu Lys Asp Arg Val Phe Ser Ser Pro  
420 425

<210> 75  
<211> 854  
<212> PRT  
<213> Homo sapiens

<400> 75

Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Pro Ser Gly  
1 5 10 15

Glu Lys Lys Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Ala Pro  
20 25 30

Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu Ala Pro  
35 40 45

Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala Gly Ala  
50 55 60

Gly Lys Pro Pro Lys Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe  
65 70 75 80

Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile Tyr His  
85 90 95

SequencesSSCPre-file7August03.ST25.txt

Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
100 105 110

Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile  
115 120 125

Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg  
130 135 140

Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg  
145 150 155 160

Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu  
165 170 175

Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe  
180 185 190

Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met  
195 200 205

Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val  
210 215 220

Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile Gly Phe  
225 230 235 240

Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu Lys Gly  
245 250 255

Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu  
260 265 270

Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln Thr Trp  
275 280 285

Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val Ser Phe  
290 295 300

Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val  
305 310 315 320

SequencesSSCPre-file7August03.ST25.txt

Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala  
325 330 335

Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn Leu Ser  
340 345 350

Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Arg Thr Val Thr  
355 360 365

Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser Arg Leu  
370 375 380

Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys Ser Lys  
385 390 395 400

Ser Gly Leu Ala Phe Arg Lys Asp Pro Pro Pro Glu Pro Ser Pro Ser  
405 410 415

Gln Lys Val Ser Leu Lys Asp Arg Val Phe Ser Ser Pro Arg Gly Val  
420 425 430

Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr Val Arg Arg Ser  
435 440 445

Pro Ser Ala Asp Gln Ser Leu Glu Asp Ser Pro Ser Lys Val Pro Lys  
450 455 460

Ser Trp Ser Phe Gly Asp Arg Ser Arg Ala Arg Gln Ala Phe Arg Ile  
465 470 475 480

Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala Ser Leu Pro Gly  
485 490 495

Glu Asp Ile Val Asp Asp Lys Ser Cys Pro Cys Glu Phe Val Thr Glu  
500 505 510

Asp Leu Thr Pro Gly Leu Lys Val Ser Ile Arg Ala Val Cys Val Met  
515 520 525

Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu Ser Leu Arg Pro Tyr  
530 535 540

SequencesSSCPre-file7August03.ST25.txt

Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu Asp Met  
545 550 555 560

Leu Ser Arg Ile Lys Ser Leu Gln Ser Ser Val Asp Gln Ile Val Gly  
565 570 575

Arg Gly Pro Ala Ile Thr Asp Lys Asp Arg Thr Lys Gly Pro Ala Glu  
580 585 590

Ala Glu Leu Pro Glu Asp Pro Ser Met Met Gly Arg Leu Gly Lys Val  
595 600 605

Glu Lys Gln Val Leu Ser Met Glu Lys Lys Leu Asp Phe Leu Val Asn  
610 615 620

Ile Tyr Met Gln Arg Met Gly Ile Pro Pro Thr Glu Thr Glu Ala Tyr  
625 630 635 640

Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro Tyr His Ser Pro Glu  
645 650 655

Asp Ser Arg Glu His Val Asp Arg His Gly Cys Ile Val Lys Ile Val  
660 665 670

Arg Ser Ser Ser Ser Thr Gly Gln Lys Asn Phe Ser Ala Pro Pro Ala  
675 680 685

Ala Pro Pro Val Gln Cys Pro Pro Ser Thr Ser Trp Gln Pro Gln Ser  
690 695 700

His Pro Arg Gln Gly His Gly Thr Ser Pro Val Gly Asp His Gly Ser  
705 710 715 720

Leu Val Arg Ile Pro Pro Pro Ala His Glu Arg Ser Leu Ser Ala  
725 730 735

Tyr Gly Gly Gly Asn Arg Ala Ser Met Glu Phe Leu Arg Gln Glu Asp  
740 745 750

Thr Pro Gly Cys Arg Pro Pro Glu Gly Thr Leu Arg Asp Ser Asp Thr



755

760

765

Ser Ile Ser Ile Pro Ser Val Asp His Glu Glu Leu Glu Arg Ser Phe  
 770 775 780

Ser Gly Phe Ser Ile Ser Gln Ser Lys Glu Asn Leu Asp Ala Leu Asn  
 785 790 795 800

Ser Cys Tyr Ala Ala Val Ala Pro Cys Ala Lys Val Arg Pro Tyr Ile  
 805 810 815

Ala Glu Gly Glu Ser Asp Thr Asp Ser Asp Leu Cys Thr Pro Cys Gly  
 820 825 830

Pro Pro Pro Arg Ser Ala Thr Gly Glu Gly Pro Phe Gly Asp Val Gly  
 835 840 845

Trp Ala Gly Pro Arg Lys  
 850

<210> 76  
 <211> 854  
 <212> PRT  
 <213> Homo sapiens  
 <400> 76

Met Val Gln Lys Ser Arg Asn Gly Gly Val Tyr Pro Gly Pro Ser Gly  
 1 5 10 15

Glu Lys Lys Leu Lys Val Gly Phe Val Gly Leu Asp Pro Gly Ala Pro  
 20 25 30

Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu Ala Pro  
 35 40 45

Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala Gly Ala  
 50 55 60

Gly Lys Pro Pro Lys Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe  
 65 70 75 80

Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile Tyr His

Ala Tyr Val Phe Leu Leu Val Phe Ser Cys Leu Val Leu Ser Val Phe  
100 105 110

Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile  
115 120 125

Leu Glu Ile Val Thr Ile Val Val Phe Gly Val Glu Tyr Phe Val Arg  
130 135 140

Ile Trp Ala Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Arg Gly Arg  
145 150 155 160

Leu Lys Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Ile Met Val Leu  
165 170 175

Ile Ala Ser Ile Ala Val Leu Ala Ala Gly Ser Gln Gly Asn Val Phe  
180 185 190

Ala Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met  
195 200 205

Ile Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val  
210 215 220

Val Tyr Ala His Ser Lys Glu Leu Val Thr Ala Trp Tyr Ile Gly Phe  
225 230 235 240

Leu Cys Leu Ile Leu Ala Ser Phe Leu Val Tyr Leu Ala Glu Lys Gly  
245 250 255

Glu Asn Asp His Phe Asp Thr Tyr Ala Asp Ala Leu Trp Trp Gly Leu  
260 265 270

Ile Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Tyr Pro Gln Thr Trp  
275 280 285

Asn Gly Arg Leu Leu Ala Ala Thr Phe Thr Leu Ile Gly Val Ser Phe  
290 295 300

SequencesSSCPre-file7August03.ST25.txt

Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val  
305 310 315 320

Gln Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala  
325 330 335

Ala Gly Leu Ile Gln Ser Ala Trp Arg Phe Tyr Ala Thr Asn Leu Ser  
340 345 350

Arg Thr Asp Leu His Ser Thr Trp Gln Tyr Tyr Glu Arg Thr Val Thr  
355 360 365

Val Pro Met Tyr Ser Ser Gln Thr Gln Thr Tyr Gly Ala Ser Arg Leu  
370 375 380

Ile Pro Pro Leu Asn Gln Leu Glu Leu Leu Arg Asn Leu Lys Ser Lys  
385 390 395 400

Ser Gly Leu Ala Phe Arg Lys Asp Pro Pro Pro Glu Pro Ser Pro Ser  
405 410 415

Gln Lys Val Ser Leu Lys Asp Arg Val Phe Ser Ser Pro Arg Gly Val  
420 425 430

Ala Ala Lys Gly Lys Gly Ser Pro Gln Ala Gln Thr Val Arg Arg Ser  
435 440 445

Pro Ser Ala Asp Gln Ser Leu Glu Asp Ser Pro Ser Lys Val Pro Lys  
450 455 460

Ser Trp Ser Phe Gly Asp Arg Ser Arg Ala Arg Gln Ala Phe Arg Ile  
465 470 475 480

Lys Gly Ala Ala Ser Arg Gln Asn Ser Glu Glu Ala Ser Leu Pro Gly  
485 490 495

Glu Asp Ile Val Asp Asp Lys Ser Cys Pro Cys Glu Phe Val Thr Glu  
500 505 510

Asp Leu Thr Pro Gly Leu Lys Val Ser Ile Arg Ala Val Cys Val Met  
515 520 525

SequencesSSCPre-file7August03.ST25.txt

Arg Phe Leu Val Ser Lys Arg Lys Phe Lys Glu Ser Leu Arg Pro Tyr  
530 535 540

Asp Val Met Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu Asp Met  
545 550 555 560

Leu Ser Arg Ile Lys Ser Leu Gln Ser Arg Val Asp Gln Ile Val Gly  
565 570 575

Arg Gly Pro Ala Ile Thr Asp Lys Asp Arg Thr Lys Gly Pro Ala Glu  
580 585 590

Ala Glu Leu Pro Glu Asp Pro Ser Met Met Gly Arg Leu Gly Lys Val  
595 600 605

Glu Lys Gln Val Leu Ser Met Glu Lys Lys Arg Asp Phe Leu Val Asn  
610 615 620

Ile Tyr Met Gln Arg Met Gly Ile Pro Pro Thr Glu Thr Glu Ala Tyr  
625 630 635 640

Phe Gly Ala Lys Glu Pro Glu Pro Ala Pro Pro Tyr His Ser Pro Glu  
645 650 655

Asp Ser Arg Glu His Val Asp Arg His Gly Cys Ile Val Lys Ile Val  
660 665 670

Arg Ser Ser Ser Ser Thr Gly Gln Lys Asn Phe Ser Ala Pro Pro Ala  
675 680 685

Ala Pro Pro Val Gln Cys Pro Pro Ser Thr Ser Trp Gln Pro Gln Ser  
690 695 700

His Pro Arg Gln Gly His Gly Thr Ser Pro Val Gly Asp His Gly Ser  
705 710 715 720

Leu Val Arg Ile Pro Pro Pro Pro Ala His Glu Arg Ser Leu Ser Ala  
725 730 735

Tyr Gly Gly Gly Asn Arg Ala Ser Met Glu Phe Leu Arg Gln Glu Asp  
740 745 750

SequencesSSCPre-file7August03.ST25.txt

Thr Pro Gly Cys Arg Pro Pro Glu Gly Thr Leu Arg Asp Ser Asp Thr  
755 760 765

Ser Ile Ser Ile Pro Ser Val Asp His Glu Glu Leu Glu Arg Ser Phe  
770 775 780

Ser Gly Phe Ser Ile Ser Gln Ser Lys Glu Asn Leu Asp Ala Leu Asn  
785 790 795 800

Ser Cys Tyr Ala Ala Val Ala Pro Cys Ala Lys Val Arg Pro Tyr Ile  
805 810 815

Ala Glu Gly Glu Ser Asp Thr Asp Ser Asp Leu Cys Thr Pro Cys Gly  
820 825 830

Pro Pro Pro Arg Ser Ala Thr Gly Glu Gly Pro Phe Gly Asp Val Gly  
835 840 845

Trp Ala Gly Pro Arg Lys  
850